



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> CHIMERIC NEOMORPHOGENESIS OF ORGANS BY CONTROLLED CELLULAR IMPLANTATION USING ARTIFICIAL MATRICES  <b>(57) Abstract</b>  A method and means for providing functional equivalents to organs wherein cells are grown on polymer scaffolding using cell culture techniques followed by transfer of the polymer-cell scaffold into a patient at a site appropriate for attachment, growth and function, after growth and vascularization. Once the structure is implanted and vascularization takes place, the resulting organ is a blend of the parenchymal elements of the donated tissue and vascular and matrix elements of the host. A key element of the method is the design and construction of the polymer scaffold using a material and shape that provides for attachment and growth of the cells such that an adequate exchange of nutrients, wastes and gases occurs by diffusion even within the inner layers of the cells, until such time as implantation and vascularization occur.		

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CHIMERIC NEOMORPHOGENESIS OF ORGANS BY CONTROLLED  
CELLULAR IMPLANTATION USING ARTIFICIAL MATRICES

1 Background of the Invention

2 This is a continuation-in-part of U.S. Serial  
3 No. 933,018 entitled "Chimeric Neomorphogenesis of  
4 Organs Using Artificial Matrices" filed November 20,  
5 1986 by Joseph P. Vacanti and Robert S. Langer.

6 The United States Government has rights in this  
7 invention by virtue of NIH grant No. 6M 26698.

8 This invention is generally in the field of  
9 medicine and cell culture, and in particular in the  
10 area of implantable organs formed on biocompatible  
11 artificial matrices.

12 Loss of organ function can result from  
13 congenital defects, injury or disease.

14 One example of a disease causing loss of organ  
15 function is diabetes mellitus. Diabetes mellitus  
16 destroys the insulin producing beta cells of the  
17 pancreas. As a consequence, serum glucose levels rise  
18 to high values because glucose cannot enter cells to  
19 meet their metabolic demands. Through a complex  
20 series of events, major problems develop in all  
21 systems secondary to the vascular changes which occur.  
22 The current method of treatment consists of the  
23 exogenous administration of insulin, which results in  
24 imperfect control of blood sugar levels. The degree  
25 of success in averting the complications of diabetes  
26 remains controversial.

27 A recent and still experimental approach has  
28 been the transplantation of pancreatic tissue, either  
29 as a whole organ or as a segment of an organ, into the  
30 diabetic patient. Serum glucose appears to be  
31 controlled in a more physiological manner using this  
32 technique and the progression of complications is  
33 thereby slowed. An earlier approach which was not  
34 successful in achieving long-term benefits was the

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1 transplantation of islet cells through injection of  
2 isolated clusters of islet cells into the portal  
3 circulation, with implantation in the vascular bed of  
4 the liver. More recent experimental methods have  
5 included encapsulation of pancreatic beta cells to  
6 prevent immune attack by the host and injection of  
7 fetal beta cells beneath the capsule of the kidney.  
8 Although there is evidence of short term function,  
9 long term results have been less satisfactory (D.E.R.  
10 Sutherland, Diabetologia 20, 161-185 (1981); D.E.R.  
11 Sutherland, Diabetologia 20, 435-500 (1981)). Currently  
12 whole organ pancreatic transplantation is the  
13 preferred treatment.

14 There are also many diseases which cause  
15 significant scarring of the liver, ultimately causing  
16 hepatic failure. There are no artificial support  
17 systems for liver failure, so that, in the absence of  
18 a successful transplant, liver failure always results  
19 in the death of the patient. It has been estimated  
20 that 30,000 people die of hepatic failure every year  
21 in the United States, at a cost to society of \$14  
22 billion dollars annually.

23 There are many diseases which are termed "inborn  
24 errors of metabolism", including genetic defects that  
25 result in defects of protein metabolism, defects of  
26 amino acid metabolism, defects of carbohydrate  
27 metabolism, defects of pyrimidine and purine  
28 metabolism, defects of lipid metabolism, and defects  
29 of mineral metabolism. A large number of these  
30 diseases are based in defects within the liver itself.  
31 Many of these patients have a structurally normal  
32 liver or reasonably normal liver at the time diagnosis  
33 is made. Many of the diseases, in fact, do not damage

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1 the native liver, rather, the damage occurs in other  
2 organs, such as the central nervous system.

3 The usual indications for liver transplantation  
4 include acute fulminant hepatic failure, chronic  
5 active hepatitis, biliary atresia, idiopathic  
6 cirrhosis, primary biliary cirrhosis, sclerosing  
7 cholangitis, inborn errors of metabolism, some forms  
8 of malignancy, and some other rare indications. The  
9 only method for treating these patients is to maintain  
10 them until a liver becomes available for  
11 transplantation. Transplantation of the whole liver  
12 has become an increasingly successful surgical  
13 manipulation through the 1980's, largely through the  
14 efforts of Dr. Thomas Starzl. However, the technical  
15 complexity of the surgery, the enormous loss of blood,  
16 the stormy postoperative course, and the many unknowns  
17 of hepatic transplantation, have made it an expensive  
18 technology available only in major medical centers.  
19 It has become increasingly clear that because of donor  
20 scarcity, transplantation will never meet the needs of  
21 the patients who require it. Currently, approximately  
22 600 patients per year undergo hepatic transplantation.  
23 Even if that capacity were tripled, it would fall  
24 short of the 30,000 patients dying of end-stage liver  
25 disease. There currently does not exist good  
26 artificial hepatic support for patients awaiting  
27 transplantation.

28 Another group of patients suffering from liver  
29 disease are those with alcohol induced liver disease.  
30 Currently, patients with end-stage liver disease from  
31 alcohol use do not have access to transplantation.  
32 There are several reasons for this including scarcity  
33 of donor organs and noncompliance with complex care.  
34 In the U.S. alone, this patient population is very

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1 large. For example, in the Baltimore area during 1973  
2 the age adjusted incidence rates for all alcoholic  
3 liver diseases per 100,000 population over 20 years  
4 were: 36.3 for white males, 19.8 for white females,  
5 60.0 for nonwhite males, and 25.4 for nonwhite  
6 females. The morbidity for liver cirrhosis has been  
7 reported to be twenty-eight times higher among serious  
8 problem drinkers than amongst nondrinkers in a survey  
9 of factory workers. There is a direct correlation  
10 between the amount of alcohol consumed and the  
11 incidence of cirrhosis. The mortality rates for  
12 cirrhosis vary greatly from country to country,  
13 ranging from 7.5 per 100,000 in Finland to 57.2 per  
14 100,000 in France. In the U.S., the trend has been  
15 alarming in terms of increasing incidence of alcoholic  
16 cirrhosis and death. Between 1950 to 1974, deaths from  
17 cirrhosis in the U.S. increased by 71.7% while deaths  
18 from cardiovascular diseases decreased by 2%. At this  
19 time, these patients have no options.

20 There are many other vital organ systems for  
21 which there is no adequate means for replacement or  
22 restoration of lost function. For example, in the  
23 past, loss of the majority of intestine was a fatal  
24 condition. Although patients can now be supported  
25 totally with nutrition supplied via the veins, this is  
26 thought of as a "half-way technology" because of the  
27 many complications associated with this technique.  
28 One problem is that, over time, many patients on total  
29 parenteral nutrition develop irreversible liver  
30 disease and die of their liver disease. Other  
31 patients develop severe blood stream infections  
32 requiring multiple removal and replacement procedures.  
33 They may eventually lose all available veins and  
34 succumb of malnutrition or die of infection.

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1           Intestinal transplantation has been unsuccessful  
2 to date because of major biological problems due to  
3 the large numbers of lymphocytes in the intestine  
4 which are transferred to the recipients. These may  
5 produce an immunologic reaction termed "graft vs.  
6 host" disease, in which the lymphocytes from the  
7 transplanted intestine attack and eventually kill the  
8 patient.

9           Diseases of the heart and muscle are also a  
10 major cause of morbidity and mortality in this  
11 country. Cardiac transplantation has been an  
12 increasingly successful technique where heart muscle  
13 has failed, but, as in the case of liver transplants,  
14 requires a donor and the use of strong  
15 immunosuppressant drugs.

16           The emergence of organ transplantation and the  
17 science of immunobiology has allowed replacement of  
18 the kidney, heart, liver, and other organs. However,  
19 as the ability to perform these complex operations has  
20 improved, the limitations of the technology have  
21 become more evident. For example, in pediatric liver  
22 transplantation, donor scarcity has increased as more  
23 programs have opened. Only a small number of donors  
24 are available in the U.S. for 800-1,000 children/year  
25 in liver failure and those children that undergo  
26 transplantation are often so ill by the time a liver  
27 is found that the likelihood of success is diminished.  
28 The surgery is complex and usually associated with  
29 major blood loss. The preservation time is short and,  
30 therefore, results in major logistical problems in  
31 matching a distant donor with a recipient. For these  
32 reasons, the undertaking is expensive and labor  
33 intensive, requiring a major investment of resources  
34 available only in tertiary care facilities.

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1        Selective cell transplantation of only those  
2 parenchymal elements necessary to replace lost  
3 function has been proposed as an alternative to whole  
4 or partial organ transplantation (P.S.Russell, Ann.  
5 Surg. 201(3),255-262 (1985)). This has several  
6 attractive features, including avoiding major surgery  
7 with its attendant blood loss, anesthetic  
8 difficulties, and complications. It replaces only  
9 those cells which supply the needed function and,  
10 therefore, problems with passenger leukocytes, antigen  
11 presenting cells, and other cell types which may  
12 promote the rejection process are avoided. Adding the  
13 techniques of cell culture provides another set of  
14 tools to aid in the transplantation process. The  
15 ability to expand cell numbers with proliferation of  
16 cells in culture, in theory, allows  
17 autotransplantation of one's own tissue. For example,  
18 hepatocyte injections into the portal circulation have  
19 been attempted to support hepatic function. A recent  
20 novel approach in which hepatocytes were attached to  
21 collagen coated microcarrier beads prior to injection  
22 into the peritoneal cavity demonstrated successful  
23 implantation, viability of the implanted hepatocytes,  
24 and function, as described by A.A.Demetriou, et al.,  
25 Science 233,1190-1192 (1986).

26        Loss of other types of organ or tissue function  
27 such as muscle or nervous tissue can also lead to  
28 deforming illnesses and social tragedies. Methods of  
29 muscle and nerve transfer have been developed by  
30 surgeons through the last fifty years which are  
31 ingenious in design. An example of a technique for  
32 restoring nerve function has been to string dead nerve  
33 fibers from nerve centers to places with lost nerve  
34 function. Many other disorders of the nervous system



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1 have eluded adequate medical thereapy. Recently,  
2 nerve cell transplantation has been proposed as a  
3 treatment modality in certain degenerative diseases of  
4 the nervous system such as Parkinson's disease and  
5 Alzheimer's disease. Autotransplantation of the  
6 adrenal tissue or injection of fetal cell suspensions  
7 into the brain appears to be of benefit. Loss,  
8 deformation or obstruction of blood vessels is another  
9 frequent cause of disease, such as high blood pressure  
10 or aneurysm. In the past, surgeons have primarily  
11 dealt with this problem by grafting blood vessels from  
12 another portion of the body to the affected area or by  
13 implanting cloth substitutes as permanent  
14 replacements. Disadvantages include the requirement  
15 of multiple operations as well as the associated pain  
16 to the patient.

17 Even though these techniques do not have many of  
18 the problems associated with transplantation of organs  
19 such as the liver or intestine, the results are still  
20 often imperfect.

21 Although different from organs such as the liver  
22 and intesting in a number of ways, skin is also an  
23 organ subject to damage by disease or injury which  
24 performs the vital role of protecting the body from  
25 fluid loss and disease. Although skin grafts have  
26 been prepared from animal skin or the patient's skin,  
27 more recently "artificial skin" formed by culturing  
28 epidermal cells has been utilized.

29 One method for forming artificial skin is by  
30 seeding a fibrous lattice with epidermal cells. For  
31 example, U.S. Patent No. 4,485,097 to Bell discloses a  
32 hydrated collagen lattice which, in combination with  
33 contractile agents such as platelets and fibroblasts  
34 and cells such as keratinocytes, is used to produce a

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1 skin-equivalent. U.S. Patent No. 4,060,081, to Yannas  
2 et al. discloses a multilayer membrane useful as  
3 synthetic skin which is formed from an insoluble non-  
4 immunogenic material which is nondegradable in the  
5 presence of body fluids and enzymes, such as cross-  
6 linked composites of collagen and a  
7 mucopolysaccharide, overlaid with a non-toxic material  
8 such as a synthetic polymer for controlling the  
9 moisture flux of the overall membrane. U.S. Patent  
10 No. 4,458,678 to Yannas et al. discloses a process for  
11 making a skin-equivalent material wherein a fibrous  
12 lattice formed from collagen cross-linked with  
13 glycosaminoglycan is seeded with epidermal cells.

14 A disadvantage to the first two methods is that  
15 the matrix is formed of a "permanent" synthetic  
16 polymer. The '678 patent has a feature that neither  
17 of the two prior patents has, a biodegradable matrix  
18 which can be formed of any shape, using the  
19 appropriate cells to produce an organ such as the  
20 skin. Unfortunately, there is a lack of control over  
21 the composition and configuration of the latter  
22 matrices since they are primarily based on collagen.  
23 Further, since collagen is degraded by enzymatic  
24 action as well as over time by hydrolysis, the  
25 degradation is quite variable. Moreover, the matrix  
26 is completely infiltrated with cells and functional in  
27 the absence of the moisture controlling polymer  
28 overlay only when it is grafted onto the patient and  
29 capillaries have formed a vascular network through the  
30 entire thickness of the matrix. The limitation of  
31 these matrices as a function of diffusion is discussed  
32 in the article by Yannas and Burke in  
33 J.Biomed.Mater.Res., 14, 65-81 (1980) at page 73.  
34 Although the authors recognized that the pore size and

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1 thickness of the matrix were controlling factors in  
2 determining viability and successful engraftment,  
3 their only ways of dealing with the lack of sufficient  
4 nutrient supply to the interior portions of the matrix  
5 at the time of engraftment were either to ignore the  
6 problem and hope the graft was thin enough and porous  
7 enough to allow sufficient capillary growth along with  
8 migration of the epithelial cells into the matrix, or  
9 to seed the graft with additional epithelial cells  
10 after sufficient capillary growth into the matrix had  
11 occurred.

12 Although skin is considered to be an "organ" of  
13 the body, these methods for making artificial skin  
14 have not been used to make other types of organs such  
15 as a liver or pancreas, despite the all encompassing  
16 statements in the patents that the disclosed or  
17 similar techniques could be utilized to do so. It is  
18 postulated that, when these methods are used to  
19 construct organs having a larger overall three  
20 dimensional structure, such as a liver or pancreas,  
21 the cells within the center of the organs tend to die  
22 after a period of time and that the initial growth  
23 rate is not maintained, in a manner analogous to the  
24 situation with very large tumors which are internally  
25 necrotic due to a decrease in diffusion of nutrients  
26 into the growing three-dimensional structure as the  
27 cell density and thickness increase. Indeed, in view  
28 of the Yannas and Burke article, it appears that  
29 growth within a matrix, even one as thin as a skin  
30 graft, presented problems until vascularization had  
31 occurred, even at relatively low cell densities.

32 It is therefore an object of the present  
33 invention to disclose a method and means for creating  
34 a variety of organs, including skin, liver, kidneys,

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1 blood vessels, nerves, and muscles, which functionally  
2 resemble the naturally occurring organ.

3 It is a further object of the present invention  
4 to provide a method and means for designing,  
5 constructing and utilizing artificial matrices as  
6 temporary scaffolding for cellular growth and  
7 implantation.

8 It is a still further object of the invention to  
9 provide biodegradable, non-toxic matrices which can be  
10 utilized for cell growth, both in vitro and in vivo,  
11 as support structures in transplant organs immediately  
12 following implantation.

13 It is another object of the present invention to  
14 provide a method for configuring and constructing  
15 biodegradable artificial matrices such that they not  
16 only provide a support for cell growth but allow and  
17 enhance vascularization and differentiation of the  
18 growing cell mass following implantation.

19 It is yet another object of the invention to  
20 provide matrices in different configurations so that  
21 cell behavior and interaction with other cells, cell  
22 substrates, and molecular signals can be studied in  
23 vitro.

#### 24 Summary of the Invention

25 The present invention is a method and means  
26 whereby cells having a desired function are grown on  
27 polymer scaffolding using cell culture techniques,  
28 followed by transfer of the polymer-cell scaffold into  
29 a patient at a site appropriate for attachment, growth  
30 and function, after attachment and equilibration, to  
31 produce a functional organ equivalent. Success  
32 depends on the ability of the implanted cells to

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1 attach to the surrounding environment and to stimulate  
2 angiogenesis. Nutrients and growth factors are  
3 supplied during cell culture allowing for attachment,  
4 survival or growth as needed.

5 After the structure is implanted and growth and  
6 vascularization take place, the resulting organoid is  
7 a chimera formed of parenchymal elements of the  
8 donated tissue and vascular and matrix elements of the  
9 host. The polymer scaffolding used for the initial  
10 cell culture is constructed of a material which  
11 degrades over time and is therefore not present in the  
12 chimeric organ. Vascular ingrowth following  
13 implantation allows for normal feedback mechanisms  
14 controlling the soluble products of the implanted  
15 cells.

16 The preferred material for forming the matrix or  
17 support structure is a biodegradable artificial  
18 polymer, for example, polyglycolic acid,  
19 polyorthoester, or polyanhydride, which is degraded by  
20 hydrolysis at a controlled rate and reabsorbed. These  
21 materials provide the maximum control of  
22 degradability, manageability, size and configuration.  
23 In some embodiments these materials are overlaid with  
24 a second material such as gelatin or agarose to  
25 enhance cell attachment. The polymer matrix must be  
26 configured to provide both adequate sites for  
27 attachment and adequate diffusion of nutrients from  
28 the cell culture to maintain cell viability and growth  
29 until the matrix is implanted and vascularization has  
30 occurred. The presently preferred structure for organ  
31 construction is a branched fibrous tree-like structure  
32 formed of polymer fibers having a high surface area.  
33 The preferred structure results in a relatively  
34 shallow concentration gradient of nutrients, wastes,

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1 and gases, so as to produce uniform cell growth and  
2 proliferation. Theoretical calculations of the  
3 maximum cell attachment suggest that fibers 30 microns  
4 in diameter and one centimeter in length can support  
5 125,000,000 cells and still provide access of  
6 nutrients to all of the cells. Another advantage of  
7 the biodegradable material is that compounds may be  
8 incorporated into the matrix for slow release during  
9 degradation of the matrix. For example, nutrients,  
10 growth factors, inducers of differentiation or de-  
11 differentiation, products of secretion,  
12 immunomodulators, inhibitors of inflammation,  
13 regression factors, biologically active compounds  
14 which enhance or allow ingrowth of the lymphatic  
15 network or nerve fibers, and drugs can be incorporated  
16 into the matrix or provided in conjunction with the  
17 matrix, in solution or incorporated into a second  
18 biodegradable polymer matrix.

19 Cells of one or more types can be selected and  
20 grown on the matrix. The matrix structure and the  
21 length of time and conditions under which the cells  
22 are cultured in vitro are determined on an individual  
23 basis for each type of cell by measuring cell  
24 attachment (only viable cells remain attached to the  
25 polymers), extent of proliferation, and percent  
26 successful engraftment. Examples of cells which are  
27 suitable for implantation include hepatocytes and bile  
28 duct cells, islet cells of the pancreas, parathyroid  
29 cells, thyroid cells, cells of the adrenal-  
30 hypothalamic-pituitary axis including hormone-producing  
31 gonadal cells, epithelial cells, nerve cells, heart  
32 muscle cells, blood vessel cells, lymphatic vessel  
33 cells, kidney cells, and intestinal cells, cells

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1 forming bone and cartilage, smooth and skeletal  
2 muscle.

3 Initially growing the cells in culture allows  
4 manipulation of the cells which may be beneficial  
5 following implantation of the matrix cell structure.  
6 Presently available technology allows the introduction  
7 of genes into the cells to make proteins which would  
8 otherwise be absent, such as those resulting from  
9 liver protein deficiencies and metabolic defects such  
10 as cystic fibrosis. Repression of gene expression may  
11 also be used to modify antigen expression on the cell  
12 surface, and thereby the immune response, so that  
13 cells are not recognized as foreign.

14 The present invention also provides techniques  
15 and matrices for in vitro studies. Although current  
16 methods of cell culture have provided valuable insight  
17 into fundamental aspects of cell organization and  
18 function, studies of cell behavior, communication,  
19 control, and morphogenesis have been difficult for  
20 lack of a system controllable in three dimensions.  
21 Artificial matrices which have been coated with  
22 attached cells can be embedded in extracellular  
23 matrices such as collagen, basement membrane complexes  
24 such as Matrigel<sup>Tm</sup>, or other materials. Various  
25 combinations of cell types, biochemical signals for  
26 growth, differentiation, migration, and extracellular  
27 matrix components can then be examined in vitro in a  
28 three-dimensional system. By controlling all of these  
29 elements, and watching behavior, the field of  
30 biomedical science may gain new insights into the  
31 actions of cells in a setting more closely resembling  
32 structure as it occurs in nature.

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1                    Brief Description of the Drawings

2            Figure 1 is a schematic of the process of the  
3 present invention to produce a chimeric organ, in this  
4 diagram, a liver, pancreas or intestine: (1) the  
5 appropriate parenchymal cells are harvested,  
6 dispersed, and seeded onto the polymer matrix in cell  
7 culture, where attachment and growth occur and (2) a  
8 partial hepatectomy is performed to stimulate growth  
9 of the transplant and the polymer-cell scaffold is  
10 then implanted into the recipient animal where  
11 neovascularization, cell growth, and reabsorption of  
12 the polymer matrix occurs.

13           Figure 2 are the chemical structures of polymers  
14 which have been used for biodegradable cellular  
15 matrices: (a) polygalactin; (b) polyorthoester; and  
16 (c) polyanhydride.

17           Figure 3 is a diagram demonstrating the slow  
18 release of biologically active factors from the  
19 polymer matrix.

20           Figure 4 is a diagram of a technique to study in  
21 vitro morphogenesis using biodegradable polymers,  
22 cells, and matrix.

23           Figure 5 is a photograph (172x) of hepatocytes  
24 attached to fibers of polyglactin 910 after 4 days in  
25 culture. Cells are stained with Hematoxylin and  
26 Eosin.

27           Figure 6 is a photograph of bile duct epithelial  
28 cells cultured on polymer fibers for one month.

29           Figure 7 is a photograph (172X) of an implant of  
30 hepatocytes from an adult rat donor into omentum. The  
31 polymer-cell implant has been in place for 7 days  
32 before sacrifice. Hepatocytes are healthy and several  
33 mitotic figures can be seen. Blood vessels are



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1 present in the mass. To the left, an inflammatory  
2 infiltrate in the area of the polymer is observed.  
3 Cells are stained with Hematoxylin and Eosin.

4 Figure 8 is a scanning electron micrograph  
5 (121X) of hepatocytes attached to polymer fibers for  
6 one week.

7 Figure 9 is a higher magnification (1600X) of  
8 the hepatocytes on polymer fibers of Figure 8.

9 Figure 10 is a photomicrograph (10X) of an  
10 intestinal cell implant into omentum ten days after  
11 implantation. It shows a 6 mm cystic structure that  
12 has formed in the omentum with blood vessels streaming  
13 into it. Polymer fibers can be seen in the wall of  
14 the cyst.

15 Figure 11 is a photograph (172X) of a cross-  
16 section of the cyst of Figure 10 demonstrating a  
17 luminal structure lined by intestinal epithelial  
18 cells. These cells show polarity. The lumen contains  
19 cellular debris and mucous. The white oval areas to  
20 the left of the lumen represent polymer fibers. They  
21 are surrounded by an inflammatory infiltrate and new  
22 blood vessels. A layer of smooth muscle can be seen  
23 to the right of the lumen, suggesting that this cyst  
24 may have arisen from a small intestinal fragment.  
25 Hematoxylin and Eosin.

26 Figure 12 is a photograph of Islets of the  
27 pancreas attached to polymer fibers after four weeks  
28 in culture, showing some secretion of insulin in  
29 response to glucose.

30 Figure 13 is a photograph of polymer fibers  
31 seeded with bovine aortic endothelial cells in a  
32 biomatrix. The cells can be seen migrating off the  
33 polymer into the matrix in a branch-like orientation.

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1        Figure 14 is a photograph of bovine aortic  
2 endothelial cells attached to polymer fibers after one  
3 month in culture.

4        Figure 15 is a phase contrast photomicrograph  
5 showing polymer fibers coated with mouse fetal  
6 fibroblasts. The fibroblasts can be seen streaming  
7 off the polymer fibers in a straight line onto the  
8 culture dish.

9        Figure 16 is a phase contrast photomicrograph of  
10 polymer fibers coated with mouse fetal fibroblasts.  
11 These fetal fibroblasts have migrated off of the  
12 polymer through media and have attached at the bottom  
13 of the tissue culture plate.

14       Figure 17 is a scanning electron micrograph  
15 (472x) of a polyanhydride fiber immersed in a  
16 phosphate buffer solution, indicating that immersion  
17 of polymer fibers in differing buffers can alter the  
18 polymer surface and, therefore, influence cell  
19 attachment and differentiation.

20       Figure 18 is a scanning electron micrograph  
21 (493x) of polymer fibers coated with 1% gelatin,  
22 showing that the polymer fibers can be coated with  
23 cell adhesion agents to increase cell attachment.

24       Figure 19 is a perspective drawing of a  
25 bioabsorbable polymer fiber used for growth of nerve  
26 cells.

27       Figure 20a is a plan drawing of polymer spicules  
28 seeded with heart muscle cells and implanted on the  
29 myocardium of the heart.

30       Figure 20b is an enlarged plan view of a  
31 spicule, as shown in Figure 20a.

32       Figure 21a is a cross sectional view of wells  
33 containing various thicknesses of collagen (0, 3.0 mm,

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1 5.5 mm, 9.0 mm, and 12.0 mm) interspersed between  
2 bovine capillary endothelial cells and the media.

3 Figure 21b is a graph of the collagen thickness  
4 (mm) versus number of cells surviving after 24 hours  
5 in the wells shown in Figure 21a.

6 Figure 22 are photographs demonstrating the  
7 effect of diffusion distance on cell viability and  
8 proliferation diagrammed in Figures 21a and 21b: (a)  
9 cells from the control well after twenty-four hours,  
10 the cell number having doubled in twenty-four hours;  
11 (b) cells overlayed with 5.5 mm of 0.32% collagen,  
12 showing that the cell viability is markedly diminished  
13 and the cell number is far less than the initial  
14 plating number; and (c) cells overlayed with 12 mm of  
15 hydrated collagen placed between media and cells,  
16 showing that all of these cells are rounded and have  
17 died.

18 Detailed Description of the Invention

19 The present invention is a method to provide  
20 functional organ equivalents using bioabsorbable  
21 artificial substrates as temporary scaffolding for  
22 cellular transfer and implantation. The success of  
23 the method depends on the integration of the following  
24 principles:

25 1. Every structure in living organisms is in a  
26 dynamic state of equilibrium, undergoing constant  
27 renewal, remodeling and replacement of functional  
28 tissue which varies from organ to organ and structure  
29 to structure.

30 2. Dissociated structural cells tend to reform  
31 structure, depending on the environment in which they

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1 are placed and the degree of alteration which they  
2 have undergone.

3       3. Tissue cannot be implanted in volumes  
4 greater than approximately one to three mm<sup>3</sup>, because  
5 nutrition is supplied by diffusion until new blood  
6 vessels form, and this distance is the maximum  
7 distance over which diffusion can transpire until  
8 angiogenesis occurs.

9       4. Cell shape is determined by cytoskeletal  
10 components and attachment to matrix plays an important  
11 role in cell division and differentiated function. If  
12 dissociated cells are placed into mature tissue as a  
13 suspension without cell attachment, they may have a  
14 difficult time finding attachment sites, achieving  
15 polarity, and functioning because they begin without  
16 intrinsic organization. This limits the total number  
17 of implanted cells which can remain viable to  
18 organize, proliferate, and function.

19       The latter principle is a key point in the  
20 configuration of the support matrices. For an organ  
21 to be constructed in tissue culture and subsequently  
22 successfully implanted, the matrices must have  
23 sufficient surface area and exposure to nutrients such  
24 that cellular growth and differentiation can occur  
25 prior to the ingrowth of blood vessels following  
26 implantation. After implantation, the configuration  
27 must allow for diffusion of nutrients and waste  
28 products and for continued blood vessel ingrowth as  
29 cell proliferation occurs.

30       This method for replacing or supplementing lost  
31 organ function has a number of advantages over either  
32 pharmacologic manipulation or transplantation of whole  
33 organs or parts of organs. Although great strides  
34 have been made in these areas, the results of these

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1 efforts are often deficient. Success in  
2 transplantation or pharmacologic manipulation may  
3 modify the outcome of a disease, but it usually does  
4 not result in cure, or it trades the original disease  
5 for the complications of non-specific  
6 immunosuppression.

7 One advantage of the present method is that it  
8 provides a means for selective transplantation of  
9 parenchymal cells which possess the necessary biologic  
10 function, without transplantation of passenger  
11 leukocytes and antigen-presenting cells. The result  
12 is greatly reduced risk of rejection of tissue without  
13 the use of drugs, especially if one is able to culture  
14 cells of the same or similar HLA tissue type. The  
15 present invention has another advantage over other  
16 means for treating organ function loss since the cells  
17 may be manipulated while in culture to introduce new  
18 genes to make absent protein products or modified to  
19 repress antigen expression on the cell surfaces so  
20 that immunosuppression is not needed when cells of the  
21 same HLA tissue type are not available. For example,  
22 a gene for insulin can be inserted into the patient's  
23 own deficient Islet cells. Other conditions can be  
24 corrected by insertion of the genes correcting Factor  
25 VIII deficiency, OTC deficiency, and disorders of  
26 carbohydrate and lipid metabolism. Techniques for the  
27 isolation, cloning and manipulation of these genes are  
28 available to those skilled in the art of genetic  
29 engineering.

30 The prospect of culturing the recipient's own  
31 cells for implantation has a further, more fundamental  
32 advantage: the elimination of the need for organ  
33 donors. For example, if a patient has lost 90% of his  
34 intestine because of ischemic damage, cells from the

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1 remaining 10% can be harvested and cultured. The  
2 cells expand in a logarithmic fashion in culture. The  
3 cells are cultured until suitable numbers of cells are  
4 achieved, the cells are grown onto the appropriate  
5 polymer scaffold, and placed back into the patient, to  
6 be allowed to vascularize, grow and function as a  
7 neointestine.

8 In the case of liver function replacement, it  
9 may be possible to construct a cell-matrix structure  
10 without the absolute need for hepatocyte proliferation  
11 in culture. This hypothesis is based on the  
12 observation that a high yield of hepatocytes can be  
13 obtained from a small piece of liver. For example, in  
14 experiments on 250 gm rats, it is known that the liver  
15 weighs approximately 12 gm. At a 90% viability rate  
16 this yields  $2.5 \times 10^8$  viable hepatocytes. It is also  
17 thought that only 10% of hepatic cell mass is  
18 necessary for cell function. Therefore, for a 250 gm  
19 rat, 1.2 gm of tissue is needed, an implant of  
20 approximately  $2.5 \times 10^7$  cells. This assumes no  
21 proliferation in vivo. Implants into children as well  
22 as adults are theoretically possible. An 8 month  
23 child has a normal liver that weighs approximately 250  
24 gm. That child would, therefore, need 25 gm of tissue  
25 from a biopsy from a parent. An adult liver weighs  
26 approximately 1500 gm, therefore, the biopsy would  
27 only be about 1.5% of his liver or  $5.0 \times 10^8$  cells.  
28 Again, this assumes no proliferation. An adult would  
29 need a larger biopsy which would yield about  $2.5 \times 10^9$   
30 cells. If these cells are attached with high  
31 efficiency and implanted, proliferation in the new  
32 host should occur. The resulting hepatic cell mass  
33 should be adequate to replace needed function.

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1           In distinct contrast to the prior art, the  
2 present method uses a temporary scaffolding for  
3 controlled growth and proliferation of cells in vitro,  
4 followed by implantation of functional cells into  
5 patients. The result is an organ which is  
6 vascularized in vivo to allow growth of the cells in a  
7 three-dimensional configuration similar to that of the  
8 organ whose function they are replacing. Both the  
9 design and construction of the scaffolding, as well as  
10 the conditions of the initial cell culture, are used  
11 to encourage cells to achieve their biological  
12 potential and replicate the ontogeny of organ  
13 formation which occurs in embryonic and fetal life.  
14 As described herein, this technique is termed chimeric  
15 neomorphogenesis.

16           The design and construction of the scaffolding  
17 is of primary importance. The matrix should be shaped  
18 to maximize surface area to allow adequate diffusion  
19 of nutrients and growth factors to the cells. The  
20 maximum distance over which adequate diffusion through  
21 densely packed cells can occur appears to be in the  
22 range of approximately 100 to 300 microns under  
23 conditions similar to those which occur in the body,  
24 wherein nutrients and oxygen diffuse from blood  
25 vessels into the surrounding tissue. The actual  
26 distance for each cell type and polymer structure must  
27 be determined empirically, measuring cell viability  
28 and function in vitro and in vivo. This determination  
29 for bovine capillary endothelial cells in combination  
30 with a collagen matrix will be described in detail in  
31 a subsequent example.

32           The cells are initially cultured using  
33 techniques known to those skilled in the art of tissue  
34 culture. Once the cells have begun to grow and cover

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1 the matrix, they are implanted in a patient at a site  
2 appropriate for attachment, growth and function. One  
3 of the advantages of a biodegradable polymeric matrix  
4 is that angiogenic and other bioactive compounds may  
5 be incorporated directly into the matrix so that they  
6 are slowly released as the matrix degrades in vivo.  
7 As the cell-polymer structure is vascularized and the  
8 structure degrades, the cells will differentiate  
9 according to their inherent characteristics. For  
10 example, cells which would normally form tubules  
11 within the body will shape themselves into structures  
12 resembling tubules and nerve cells will extend along  
13 an appropriately constructed pathway.

14 In the preferred embodiment, the matrix is  
15 formed of a bioabsorbable, or biodegradable, synthetic  
16 polymer such as a polyanhydride, polyorthoester, or  
17 polyglycolic acid, the structures of which are shown  
18 in Figure 2. In some embodiments, attachment of the  
19 cells to the polymer is enhanced by coating the  
20 polymers with compounds such as basement membrane  
21 components, agar, agarose, gelatin, gum arabic,  
22 collagens types I, II, III, IV, and V, fibronectin,  
23 laminin, glycosaminoglycans, mixtures thereof, and  
24 other materials known to those skilled in the art of  
25 cell culture. For in vitro studies, non-biodegradable  
26 polymer materials can be used, depending on the  
27 ultimate disposition of the growing cells, including  
28 polymethacrylate and silicon polymers. A non-  
29 degradable material is particularly useful when the  
30 cells are grown in culture for purposes other than  
31 transplantation, as in understanding cell to cell  
32 interaction: behavior, communication, control, and  
33 morphogenesis, since the preferred matrix structure  
34 allows for a higher immobilized cell density than can



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1 normally be achieved where nutrients are supplied  
2 solely by diffusion.

3 All polymers for use in the present invention  
4 must meet the mechanical and biochemical parameters  
5 necessary to provide adequate support for the cells  
6 with subsequent growth and proliferation. The  
7 polymers can be characterized with respect to  
8 mechanical properties such as tensile strength using  
9 an Instron tester, for polymer molecular weight by gel  
10 permeation chromatography (GPC), glass transition  
11 temperature by differential scanning calorimetry (DSC)  
12 and bond structure by infrared (IR) spectroscopy; with  
13 respect to toxicology by initial screening tests  
14 involving Ames assays and in vitro teratogenicity  
15 assays, and implantation studies in animals for  
16 immunogenicity, inflammation, release and degradation  
17 studies.

18 In in vitro cell attachment and viability can be  
19 assessed using scanning electron microscopy,  
20 histology, and quantitative assessment with  
21 radioisotopes.

22 The configuration of the polymer scaffold must  
23 have enough surface area for the cells to be nourished  
24 by diffusion until new blood vessels interdigitate  
25 with the implanted parenchymal elements to continue to  
26 support their growth, organization, and function.  
27 Polymer discs seeded with a monolayer of cells, and  
28 branching fiber networks both satisfy these needs.

29 At the present time, a fibrillar structure is  
30 preferred. The fibers may be round, scalloped,  
31 flattened, star shaped, solitary or entwined with  
32 other fibers. The use of branching fibers is based  
33 upon the same principles which nature has used to  
34 solve the problem of increasing surface area

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1 proportionate to volume increases. All multicellular  
2 organisms utilize this repeating branching structure.  
3 Branching systems represent communication networks  
4 between organs as well as the functional units of  
5 individual organs. Seeding and implanting this  
6 configuration with cells allows implantation of large  
7 numbers of cells, each of which is exposed to the  
8 environment of the host, providing for free exchange  
9 of nutrients and waste while neovascularization is  
10 achieved.

11 The method of the present invention is  
12 diagrammed in Figure 1. Cells 10 of the type required  
13 to provide the desired organ function are obtained  
14 from a donor, the recipient, or a cell culture line.  
15 A suspension 12 of, for example, liver, intestine, or  
16 pancreatic cells is prepared and seeded onto the  
17 polymer matrix 14. The cell-polymer scaffold 16 is  
18 cultured for an appropriate time under optimized  
19 conditions. The cell-polymer scaffold 16 is then  
20 implanted. In the example of an organ to provide lost  
21 liver function, the organ is implanted into the  
22 omentum adjacent the portal circulation which serves  
23 as a source of neovascularization. Optionally,  
24 partial hepatectomy is performed to stimulate cell  
25 regeneration. In addition to providing an adequate  
26 blood supply, "hepatotrophic" factors from the portal  
27 circulation aid in hepatic regeneration. It is also  
28 thought that factors such as insulin from the  
29 pancreatic blood supply specifically aid in the  
30 regenerative process. Alternatively, these factors,  
31 including nutrients, growth factors, inducers of  
32 differentiation or de-differentiation, products of  
33 secretion, immunomodulators, inhibitors of  
34 inflammation, regression factors, biologically active

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1 compounds which enhance or allow ingrowth of the  
2 lymphatic network or nerve fibers, and drugs, can be  
3 incorporated into the matrix or provided in  
4 conjunction with the matrix, as diagrammed in Figure  
5 3.

6       The branching fibers 14 shown in Fig. 1, when 30  
7 microns in diameter and 1.0 cm in length, can  
8 theoretically support 125,000,000 cells. In the  
9 example in which a liver organ is constructed, the  
10 cell populations can include hepatocytes and bile duct  
11 cells. Cells may be derived from the host, a related  
12 donor or from established cell lines. Fetal cells  
13 lines may be utilized since these cells are generally  
14 more hardy than other cell lines.

15       In one variation of the method using a single  
16 matrix for attachment of one or more cell lines, the  
17 scaffolding is constructed such that initial cell  
18 attachment and growth occur separately within the  
19 matrix for each population. Alternatively, a unitary  
20 scaffolding may be formed of different materials to  
21 optimize attachment of various types of cells at  
22 specific locations. Attachment is a function of both  
23 the type of cell and matrix composition.

24       Although the presently preferred embodiment is  
25 to utilize a single cell-matrix structure implanted  
26 into a host, there are situations where it may be  
27 desirable to use more than one cell-matrix structure,  
28 each implanted at the most optimum time for growth of  
29 the attached cells to form a functioning three-  
30 dimensional organ structure from the different cell-  
31 matrix structures. In some situations, it may be  
32 desirable to prepare the implantation site by  
33 initially exposing the cells at the site to a  
34 biodegradable polymer matrix including compounds or

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1 "de-differentiators" which induce a revision of the  
2 surrounding mesenchymal cells to become more  
3 embryonic. The implanted cell matrix structure may  
4 then develop more normally in the fetal environment  
5 than it would surrounded by more mature cells.

6 Applying the above-described techniques and  
7 materials to the design, construction and implantation  
8 of a functional liver-type organ, one would begin with  
9 long, solid fibers seeded with bile duct epithelial  
10 cells inserted into a structure seeded with  
11 hepatocytes. After implantation and degradation of  
12 the polymer, the bile duct cells would form the  
13 appropriate connections for delivery of the bile to  
14 the desired locations. Ingrowth of the vascular  
15 supply, lymphatic network and nerve fibers could be  
16 encouraged. The combination polymer-cell scaffold  
17 with both attached hepatocytes and biliary epithelial  
18 cells could be implanted into a retroperitoneal  
19 position behind the mesocolon. An extension of the  
20 biliary conduit can be tunneled through the mesocolon  
21 and into a limb of jejunum so that biliary drainage  
22 can enter into the jejunum or upper intestine. As  
23 vascularization, cell-cell reorganization and polymer  
24 resorption occur, hepatic function should be replaced  
25 and bile flow should commence and proceed into the  
26 intestine. This location has several potential  
27 advantages because of its vascular supply. It is  
28 known that "hepatotrophic" factors come from the  
29 portal circulation and supply the liver for  
30 regeneration. Angiogenesis may occur from the portal  
31 bed immediately adjacent to the pancreas, a known  
32 source of hepatotrophic factors, as the inflow to  
33 these implanted hepatocytes. The outflow may be  
34 through retroperitoneal collaterals that drain into

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1 the systemic circulation through the hemiazygous  
2 system. If this occurs, there would be portosystemic  
3 channels through the implanted hepatic cells which may  
4 allow for decompression of portal hypertension, a  
5 complication leading to gastrointestinal bleeding in  
6 patients with end-stage liver disease.

7 In the case of metabolic liver disease, where  
8 the native liver is structurally normal and can drain  
9 bile, appropriate hepatocytes on scaffolds can be  
10 placed directly into the recipient liver. This  
11 intrahepatic engraftment would occur in relation to  
12 the normal host biliary system. The native liver  
13 would then be a chimera of patient cells and donor  
14 cells draining into the patient's biliary tree.

15 For this procedure to be successful, the  
16 function of the implanted cells, both in vitro as well  
17 as in vivo, must be determined. In vivo liver  
18 function studies can be performed by placing a cannula  
19 into the recipient's common bile duct. Bile can then  
20 be collected in increments. Bile pigments can be  
21 analyzed by high pressure liquid chromatography  
22 looking for underivatized tetrapyrroles or by thin  
23 layer chromatography after being converted to  
24 azodipyrroles by reaction with diazotized  
25 azodipyrroles ethylanthranilate either with or without  
26 treatment with  $\beta$ -glucuronidase. Diconjugated and  
27 monoconjugated bilirubin can also be determined by  
28 thin layer chromatography after alkalinemethanolysis  
29 of conjugated bile pigments. In general, as greater  
30 numbers of functioning transplanted hepatocytes  
31 implant, the levels of conjugated bilirubin will  
32 increase. The same technique measuring monoconjugated  
33 and diconjugated bilirubin can be performed in vitro  
34 by testing the media for levels of these bilirubin

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1 conjugates. Analogous organ function studies can be  
2 conducted using techniques known to those skilled in  
3 the art, as required to determine the extent of cell  
4 function both in cell culture and after implantation.

5 In order to optimize conditions for forming  
6 implants, once in vitro and in vivo function has been  
7 confirmed, studies into morphogenesis of the  
8 structures can be initiated. Bile duct epithelial  
9 cells which have been harvested can be seeded onto  
10 polymer scaffolds. These scaffolds can then be  
11 reseeded with hepatocytes. The cell-cell  
12 interactions, shown schematically in Figure 4, can be  
13 monitored in vitro by time lapse video microscopy as  
14 well as histological sections for light microscopy,  
15 transmission microscopy, and scanning electron  
16 microscopy.

17 Studies using labelled glucose as well as  
18 studies using protein assays can be performed to  
19 quantitate cell mass on the polymer scaffolds. These  
20 studies of cell mass can then be correlated with cell  
21 functional studies to determine what the appropriate  
22 cell mass is.

23 The following examples demonstrate actual  
24 attachment of cell preparations to bioerodable  
25 artificial polymers in cell culture and implantion  
26 this polymer-cell scaffold into animals. Using  
27 standard techniques of cell harvest, single cells and  
28 clusters of fetal and adult rat and mouse hepatocytes,  
29 pancreatic islet cells, and small intestinal cells  
30 have been seeded onto biodegradable polymers of  
31 polyglactin 910, polyanhydrides, and polyorthoester.  
32 Sixty-five fetuses and 14 adult animals served as  
33 donors. One hundred and fifteen polymer scaffolds  
34 were implanted into 70 recipient animals: 66 seeded

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1 with hepatocytes; 23 with intestinal cells and  
2 clusters; and 26 with pancreatic islet preparations.  
3 The cells remained viable in culture, and in the case  
4 of fetal intestine and fetal hepatocytes, appeared to  
5 proliferate while on the polymer. After 4 days in  
6 culture, the cell-polymer scaffolds were implanted  
7 into host animals, either in the omentum, the  
8 interscapular fat pad, or the mesentery. In 3 cases  
9 of fetal intestinal implantation coupled with partial  
10 hepatectomy, successful engraftment occurred in the  
11 omentum, one forming a visible 6.0 mm cyst. Three  
12 cases of hepatocyte implantation, one using adult  
13 cells and two using fetal cells, have also engrafted,  
14 showing viability of hepatocytes, mitotic figures, and  
15 vascularization of the cell mass.

#### 16 Materials and Methods

##### 17 Polymers:

18 Three synthetic absorbable polymers were used to  
19 fabricate filaments and discs as matrices for cell  
20 attachment, growth, and implantation (Fig. 2).

21 1. Polyglactin. This polymer, developed as  
22 absorbable synthetic suture material, a 90:10  
23 copolymer of glycolide and lactide, is manufactured as  
24 Vicryl<sup>®</sup> braided absorbable suture (Ethicon Co.,  
25 Somerville, New Jersey) (Craig P.H., Williams J.A.,  
26 Davis K.W., et al.: A Biological Comparison of  
27 Polyglactin 910 and Polyglycolic Acid Synthetic  
28 Absorbable Sutures. Surg. 141; 1010, (1975)).

29 2. Polyorthoesters. The specific polymer used  
30 was: 3,9-bis(ethylidene-2, 4, 8, 10-tetraoxaspiro[5.5]  
31 undecane copolymer with tran-1,4-cyclohexanedimethanol  
32 and 1,6-hexandiol in a molar ratio 2:1:1, respectively  
33 (SRI, California) (Heller J., Penhale W.H., Helwing

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1 R.F., et al.: Release of Norethindrone from Polacetals  
2 and Polyorthoesters. AICHE Symposium Series, 206; 77,  
3 pp. 28-36 (1981)).  
4 3. Polyanhydride. The specific polymer used  
5 was a copolyanhydride of bis(1,4-  
6 carboxyphenoxy)propane and sebacic acid. It is  
7 biocompatible and has been used extensively in drug  
8 delivery applications (Heller J., Penhale W.H.,  
9 Helwing R.F., et al.: Release of Norethindrone from  
10 Polyacetals and Polyorthoesters. AICHE Symposium  
11 Series, 206; 77, pp. 28-36 1981; Leong K.W., D'Amore  
12 P., Marletta M., et al: Bioerodable Polyanhydrides as  
13 Drug Carrier Matrices. II. Biocompatibility and  
14 Chemical Reactivity. J. Biomed. Mat. Res. 20: 51,  
15 1986; Domb A.J., Langer R.: Polyanhydrides I.  
16 Preparation of High Molecular Weight Polyanhydrides.  
17 J. Poly. Sci., in press; Kopacek J., Ulbrich K.:  
18 Biodegradation of Biomedical Polymers. Prog. Poly.  
19 Sci 9:1, (1983, and references within).

20 Polymer Configuration:

21 The polyglycolide was used as supplied by the  
22 manufacturer. Small wafer discs or filaments of  
23 polyanhydrides and polyorthoesters were fabricated  
24 using one of the following methods:

25 A. Solvent Casting. A solution of 10% polymer  
26 in methylene chloride was cast on a branching pattern  
27 relief structure as a disc 10 mm in diameter for 10  
28 minutes at 25°C using a Carver press. After solvent  
29 evaporation, a film 0.5 mm in thickness with an  
30 engraved branching pattern on its surface was  
31 obtained.

32 B. Compression Molding. 100 mg of the polymer  
33 was pressed (30,000 psi) into a disc having a



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1 branching pattern relief, 10 mm in diameter and 0.5 mm  
2 thick.

3 C. Filament Drawing. Filaments were drawn from  
4 the molten polymer (30 microns in diameter). Small  
5 flattened 1.0 cm. tufts were used for the experiments.

6 D. Polyglactin 910. Multiple fibers of 90:10  
7 copolymer of glycolide and lactide converging to a  
8 common base were fashioned from suture material of Q-  
9 Vicryl® by fraying the braided end of the polymer.  
10 These branching fiber clusters were approximately 1.0  
11 cm. in height. The individual fibrils were 30 microns  
12 in diameter.

### 13 Animals:

14 Young adult and fetal Sprague-Dawley rats and  
15 C57 Bl/6 mice (Charles River Labs, Wilmington,  
16 Massachusetts) were used as cell donors for all  
17 experiments. The animals were housed individually,  
18 allowed access to food and water ad lib, and  
19 maintained at 12 hour light and dark intervals.  
20 Animals were anesthetised with an IP injection of  
21 pentobarbital (Abbott Labs, North Chicago, Illinois)  
22 at a dose of 0.05 mg/g and supplemented with  
23 methoxyflurane (Pitman-Moore, Inc., Washington  
24 Crossing, New Jersey) by cone administration. Fetal  
25 animals were harvested at 13, 17 and 20 days gestation  
26 for use as liver, pancreas, and intestinal donors.  
27 Young adult animals were used as liver and pancreas  
28 donors and as recipients of the cell-scaffold  
29 matrices.

### 30 Cell Harvest and Cell Culture

#### 31 Liver:

32 After the induction of anesthesia, the abdomen  
33 of young adult animals was shaved, prepped with  
34 betadine, and opened using sterile technique. The

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1 liver was isolated and after heparinization with 100  
2 U. of heparin (Elkins-Sinn, Inc., Cherry Hill, New  
3 Jersey), the portal vein was cannulated with a 23  
4 gauge plastic IV cannula (Critikon, Inc., Tampa,  
5 Florida). The inferior vena cava was transected, the  
6 liver flushed with 2-3 cc.'s of sterile saline,  
7 removed from its bed, and transferred to a sterile  
8 dish where it was perfused with an oxygenated solution  
9 of 0.025% collagenase type II (BCA/Cappel Products,  
10 West Chester, Pennsylvania) by a technique modified  
11 from Selgen (Selgen, P.O.: Preparation of Rat Liver  
12 Cells. III. Enzymatic Requirements of Tissue  
13 Dispersion. Exp. Cell. Res. 82: 391, 1973). After a  
14 20 minute perfusion, the liver was transferred to a  
15 sterile hood for cell dispersion and culture.

16 A two-step collagenase perfusion technique was  
17 utilized for hepatocyte harvest. The in vivo liver  
18 perfusion must involve a continuous flow of perfusate  
19 of 30-40 mm<sup>3</sup> per minute, rather than pulsatile  
20 perfusion. Initial hepatocyte harvests yielding 2-3 x  
21 10<sup>6</sup> cells with a 10-20% cell viability were improved  
22 to yield a 4-6 x 10<sup>8</sup> cell harvest with a cell  
23 viability of 80-90% by switching to a peristaltic pump  
24 which provides a continuous flow. Various buffers  
25 have also been tested for their effect. For example,  
26 HEPES' buffer was used to decrease the acidity of the  
27 perfusate.

28 To avoid contamination of the hepatocyte polymer  
29 scaffolds in culture with either fungus or bacteria,  
30 sterile technique was used both for isolation and  
31 perfusion of hepatocytes. Antibiotics were also added  
32 to the collagenase perfusion solution.

33 Fetal animals were harvested by isolating and  
34 removing the gravid uterine horns from pregnant

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1 animals of the appropriate gestation. The intact  
2 uterus with multiple fetuses was transferred in saline  
3 to a sterile room, equipped with a dissection  
4 microscope. Individual fetuses were opened and the  
5 liver, intestine, and pancreas were harvested and  
6 pooled. Organs were then transferred to a sterile  
7 hood for cell isolation. The tissues were minced,  
8 treated with a 0.025% Type II collagenase, and  
9 dispersed into cell suspensions.

10 Pancreas:

11 After the induction of anesthesia, the abdomen  
12 of young adult animals was shaved, prepped with  
13 betadine, and opened in the midline using sterile  
14 technique. The common bile duct was isolated, and the  
15 pancreas visualized. 2.5 cc.'s of 2.0% Type II  
16 collagenase (BCA/Cappel Products, West Chester,  
17 Pennsylvania), was infused into the pancreas by  
18 injection into the common bile duct using the  
19 technique described by Gotoh et al. (Gotoh M., Maki  
20 T., Kiyozumi T., et al.: An Improved Method of  
21 Isolation of Mouse Pancreatic Islets. Trans. 40; 4,  
22 pp. 436-438, 1985). After 5 minutes, the pancreas was  
23 transferred to a sterile hood for islet cell  
24 isolation. Briefly, the tissue was placed into a 25%  
25 Ficoll solution and layered under a discontinuous  
26 Ficoll gradient (23, 21, 11%) and centrifuged at 800 x  
27 g. for 10 minutes. Islets which aggregated at the 21-  
28 11% interface were washed with cold Hank's solution  
29 and centrifuged at 320 x g. 3 times. The islets were  
30 resuspended in RPMI 1640 (Gibco, Grand Island, New  
31 York) media supplemented with 10% fetal calf serum,  
32 and overlaid onto polymer scaffolds. Fetal animals  
33 were harvested as donors as described above.

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1 Intestine:

2 Fetal intestine was obtained as described above.

3 Polymer-Cell Scaffolds and Implantation:

4 Cells in suspension were plated onto polymer  
5 matrices at  $1 \times 10^5$  or  $1 \times 10^6$  cells/cc. They were  
6 maintained in Chee's media supplemented with 10% fetal  
7 calf serum for 3-4 days in a 10% CO<sub>2</sub> environment.  
8 Viability of cells on the scaffold immediately pre-  
9 implantation was assessed by the trypan blue exclusion  
10 method. Young adult Sprague-Dawley rats were  
11 anesthetized, shaved over the operative site and  
12 prepped with betadine.

13 The polymer-cell scaffold was implanted in one  
14 of three sites:

- 15 1) the interscapular fat pad;
- 16 2) the omentum; and
- 17 3) the bowel mesentery.

18 Most animals underwent a partial hepatectomy to  
19 stimulate cell growth. Animals were sacrificed at day  
20 3, 7, or 14 and the implants were examined  
21 histologically with hematoxylin and eosin. Polymers  
22 without cells served as controls. Polymer-cell  
23 scaffolds were examined histologically after 4 days in  
24 culture and before implantation to assess cell  
25 attachment and viability.

26 The following techniques are also used in the  
27 examination of the cell-matrix structures.

28 Immunofluorescent staining: tissue, including  
29 the cell-polymer scaffold, is frozen by immersion into  
30 isopentane liquid, stored at -70°C in a cryostat and  
31 mounted on albumin-coated slides. After thawing for  
32 15-30 minutes at room temperature, the slides are  
33 washed with phosphate-buffered saline (PBS). Several  
34 drops of appropriately diluted, commercially prepared

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1 fluoresceine isothiocyanate (FITC) antisera labelled to  
2 the appropriate monoclonal antibody, for example, the  
3 HY antigen or other markers of hepatocyte membranes,  
4 are applied individually to separate moist biopsy  
5 sections. They are incubated at room temperature for  
6 30 minutes in a moist chamber. Following rinses with  
7 PBS the sections are cover-slipped with a glycerol-PBS  
8 mixture and examined using an immunofluorescence  
9 microscope (Leitz) with epi-illumination and a high  
10 pressure mercury lamp as the light source.

11       Electron microscopy:       samples for electron  
12 microscopy are obtained from fresh tissue and fixed in  
13 2% glutaraldehyde, post-fixed in 1% osmium tetroxide,  
14 dehydrated in graded alcohols, and imbedded in epon-  
15 8:12. One micron thick section of the plastic  
16 imbedded tissue are made from areas of interest.  
17 Selected blocks are trimmed, ultrathin sections made,  
18 and stained with uranyl acetate and lead citrate, and  
19 examined with a Phillips 300X electron microscope.  
20 Scanning electron microscopy (SEM): After hepatocytes  
21 are isolated and attached to the appropriate polymer,  
22 they are incubated for the appropriate interval.  
23 After culture, samples are prepared for SEM by  
24 incubating in a 50:50 solution of 2% glutaraldehyde  
25 phosphate buffer solution for 1 hour, the samples are  
26 then rinsed 4 times in PBS for 10 minutes per rinse to  
27 remove excess glutaraldehyde solution. Samples are  
28 dehydrated using progressively increasing ethanol  
29 solutions. Samples are then placed in a critical  
30 point dryer where ethanol is exchanged for liquid CO<sub>2</sub>.  
31 Temperature is gradually increased to the critical  
32 point, ensuring dehydration. The samples are then  
33 coated with a thin layer of gold and placed under high  
34 vacuum in the scanning electron microscope.

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1        Seventy-nine animals which included 14 adults  
2 and 65 fetuses were used as donors for cell harvest;  
3 115 polymer scaffolds were prepared for implantation.  
4 Sixty-six of these scaffolds were seeded with  
5 hepatocytes, 23 with intestinal cells and clusters,  
6 and 26 with pancreatic islets and cell preparations.  
7 Implantation was performed in 70 recipient animals.  
8 Fifty-eight were sacrificed at 7 days for histologic  
9 examination of the implant while 3 were examined at 3  
10 days, and 9 at 14 days after implantation.

11       Cell viability on the polymer scaffold at 3 to 4  
12 days in culture varied with the type of polymer  
13 material used. Figure 5 shows hepatocytes on polymer  
14 matrices for four days. Figure 6 shows bile duct  
15 epithelial cells on polymer fibers for one month.  
16 Figures 8 and 9 show hepatocytes attached to polymer  
17 fibers for one week. Less than 10% of the cells were  
18 viable on the polyanhydride discs, whereas 80% of  
19 cells cultured on polyorthoester discs and filaments  
20 remained viable, and over 90% survived on polyglactin  
21 910.

22       Hepatocytes placed on polygalactin fibers for  
23 three weeks in culture showed evidence of significant  
24 proliferation with nodule formation one to three mm in  
25 diameter with fragmented fibers interspersed within  
26 the cell mass.

27       Blood vessel ingrowth was noted three days after  
28 implantation with all of the polymer types and  
29 configurations. In the implanted fiber networks, new  
30 blood vessels formed in the interstices between the  
31 polymer filaments. The polymer discs showed capillary  
32 formation immediately adjacent to the polymer  
33 material. This angiogenic response accompanied an  
34 inflammatory infiltrate which displayed both an acute

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1 phase and a chronic foreign body reaction to the  
2 implanted polymers. The intensity of inflammation  
3 varied with the polymer type tested: polyanhydride  
4 elicited the most severe acute and chronic response  
5 although the inflammation surrounding branching fibers  
6 of either polyorthoester or polyglactin appeared  
7 proportionately greater than the disc configuration  
8 because of the greater surface area of exposed foreign  
9 material to host.

10       Histologic examination of liver cell implants in  
11 3 animals showed evidence of successful engraftment of  
12 hepatocytes at seven days, as shown by Figure 7.  
13 Small clusters of healthy appearing hepatocytes were  
14 seen with bile canaliculi between adjacent cell  
15 membranes and some areas demonstrated mitotic figures.  
16 The cells were surrounded by an inflammatory response  
17 and blood vessels coursed around and through the cell  
18 clusters. Polymer material was seen immediately  
19 adjacent to the cells.

20       Successful engraftment of intestinal cells and  
21 clusters were observed in 3 animals. Histologic  
22 findings were similar to the hepatocyte implants. On  
23 gross examination of the implant at 7 days, a cystic  
24 structure approximately 6.0 mm in length was found at  
25 the implant site with polymer fibers displayed within  
26 its wall (Figure 10). Microscopic examination  
27 revealed well differentiated intestinal epithelium  
28 lining the cavity with mucous and cellular debris  
29 within the lumen, shown in Figure 11. One wall of the  
30 cyst contained polymer fibers, blood vessels, and  
31 inflammatory cells immediately adjacent to the  
32 intestinal epithelium. The other wall included a  
33 muscular coating which suggested that the polymer held  
34 a small minced piece of fetal intestine as the origin

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1 of the cyst which eventually developed. The cyst  
2 displayed well differentiated intestinal epithelium  
3 with mucous secreting cells. Other clusters of  
4 intestinal epithelium demonstrated active mitosis.

5 Control polymers implanted without prior cell  
6 seeding elicited an angiogenic and inflammatory  
7 response similar to their counterparts which had been  
8 seeded with parenchymal cells and maintained in  
9 culture. This suggested that the cells themselves did  
10 not play a major role in the inflammation and  
11 neovascularization seen. If appropriate,  
12 immunosuppressant drugs may be injected or  
13 incorporated into the polymer structure. However, a  
14 limited inflammatory reaction to the implant may in  
15 fact be desirable to promote growth. This encourages  
16 a more normal healing response and may play a role in  
17 the "calling in" of new blood vessels.

18 The use of the donor's own cells or cells from  
19 which the lymphocytes have been removed prior to  
20 culturing is especially important in the culturing and  
21 implantation of intestinal cells. If the lymphocytes  
22 are not removed from the intestinal cells prior to  
23 implantation, the result can be "graft vs. host"  
24 disease. The present invention decreases this  
25 possibility since only the cells needed for function  
26 are placed on the polymers and implanted into the  
27 patient.

28 Other types of cells which have been  
29 successfully cultured and demonstrated to retain  
30 function include pancreatic cells and aortic cells.  
31 Figure 12 is a photograph of Islets of the pancreas  
32 attached to polymer fibers after four weeks in  
33 culture, showing some secretion of insulin in response  
34 to glucose. Figure 13 is a photograph of polymer



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1 fibers seeded with bovine aortic endothelial cells in  
2 a biomatrix. The cells can be seen migrating off the  
3 polymer into the matrix in a branch-like orientation.  
4 Figure 14 is a photograph of bovine aortic endothelial  
5 cells attached to polymer fibers after one month in  
6 culture. These cells have been shown to reform  
7 structure. Their ability to do so depends upon the  
8 environment in which they are placed and the degree of  
9 alteration they have undergone. In addition to the  
10 the bile duct cells which formed tubules in vitro as  
11 shown in Figure 6, the aortic endothelial cells  
12 attached to polymer fibers formed branching tubule  
13 structures after one month in culture. As the polymer  
14 fibers resorbed, the cells maintained their  
15 orientation, indicating that they secreted their own  
16 matrix to maintain their geometric configuration.

17 Figure 15 is a phase contrast photomicrograph  
18 showing polymer fibers coated with mouse fetal  
19 fibroblasts. The fibroblasts can be seen streaming  
20 off the polymer fibers in a straight line onto the  
21 culture dish. This indicates that cell-cell  
22 orientation cues have been maintained as they migrate  
23 off the polymer fiber.

24 Figure 16 is a phase contrast photomicrograph of  
25 polymer fibers coated with mouse fetal fibroblasts.  
26 These fetal fibroblasts have migrated off of the  
27 polymer through media and have attached at the bottom  
28 of the tissue culture plate. This shows that a living  
29 tissue bridge has been created between the polymer  
30 fiber and the tissue culture bottom by fetal  
31 fibroblasts, indicating their spatial organization.

32 These studies demonstrate that cells of liver,  
33 intestine, and pancreas will successfully attach and  
34 remain viable on polymers in cell culture and that

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1 liver and intestinal cells will successfully engraft  
2 in a host animal. The following methods were used to  
3 demonstrate the optimization of cell attachment to  
4 polymers, using liver and pancreas as model systems.

5 A nonquantitative cell attachment study was  
6 undertaken in which N.I.H. 3T3 cells were used as  
7 model cells for attachment studies. Polymers tested  
8 included polyglactin, polyorthoester, and  
9 polyanhydride. Attachment studies were then performed  
10 on pancreatic islets.

11 Polymer Preparation.

12 Polyglactin 910, polyorthoester, and  
13 polyanhydrides were treated with several different  
14 buffers in an effort to change the surface  
15 conformation of the polymer, and were coated with  
16 various materials thought to be important for cell  
17 attachment. Each polymer was tested by soaking in a  
18 citric acid buffer solution, pH 4.0, phosphate buffer  
19 solution, pH 7.0, or a carbonate buffer, pH 10.0.  
20 These were incubated at 37°C for 2, 5, or 7 days.  
21 Surface characteristics of the polymer material were  
22 characterized by scanning electron microscopy (SEM) at  
23 magnifications of 500X and 1700X.

24 Different coatings included: agar at 2% and 5%  
25 solutions, agarose at 2%, 6%, and 7% solutions,  
26 gelatin at 1.5% and 11% and gum arabic at 1.5% and  
27 11%. Coatings were prepared by making a solution of  
28 the appropriate weight of material in deionized water  
29 and autoclaving for 30 minutes. Solutions were  
30 maintained in the liquid state in a warm water bath at  
31 40-50°C until used. Using sterile technique, each  
32 polymer was immersed into the appropriate coating  
33 material. Gelatin was cross-linked with a 50:50  
34 solution of 2% gluteraldehyde:phosphate buffer for 1

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1 hour. A combined coating using gelatin and gum arabic was tested. Collagen coated polymers were prepared by covering the polymer with a Type IV collagen and lyophilizing this polymer-collagen material overnight. Some collagen coated samples were immersed in phosphate buffer for one hour. All samples were examined by SEM to determine uniformity of coating. All samples were sterilized using UV exposure under the sterile hood for 8-12 hours. Cells were then added for cell attachment studies.

#### 11 Cell Attachment Studies.

12 Cell polymer samples were examined by phase contrast microscopy and SEM using the following sample preparation technique. Samples were fixed by immersion in 50:50 2% gluteraldehyde:phosphate buffer for 1 hour and then rinsed x 3 for 20 minutes with phosphate buffer. They were then dehydrated in progressively increasing concentrations of ethanol solutions (70%, 80%, 90%, 95%) for 20 minutes each, immersed in absolute alcohol overnight, dried by critical point drying with liquid CO<sub>2</sub> and coated with gold.

#### 23 Isolation and purification of Pancreatic Islet Cells.

24 Young adult mice were anesthetized and underwent a midline abdominal incision using sterile technique. The common bile duct was isolated and cannulated with a 30 gauge needle. 2.5 cc. of Type IV collagenase was slowly infused through the common bile duct with a clamp on the duodenum so that there would be retrograde flow into the pancreatic duct. The pancreas was then removed and digested with collagenase for 45 minutes at 37°C. The pancreas was then washed with cold Hank's solution and pancreatic tissue passed through a nylon mesh filter. The islets

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1 were then isolated using a discontinuous Ficoll  
2 gradient. then washed with cold Hank's solution and  
3 resuspended in RPMI 1640 media enriched with 10% fetal  
4 calf serum. Islets were placed in 24 well plates on  
5 the appropriate polymer and incubated at 37°, 10% CO<sub>2</sub>.

6 N.I.H. 3T3 cells were used as a cell line for  
7 other attachment studies.

8 Figure 17 is a scanning electron micrograph  
9 (472x) of a polyanhydride fiber immersed in a  
10 phosphate buffer solution, indicating that immersion  
11 of polymer fibers in differing buffers can alter the  
12 polymer surface and, therefore, influence cell  
13 attachment and differentiation.

14 Figure 18 is a scanning electron micrograph  
15 (493x) of polymer fibers coated with 1% gelatin,  
16 showing that the polymer fibers can be coated with  
17 known cell adhesion agents to increase cell  
18 attachment.

19 Table I is the attachment of 3T3 cells on  
20 Vicryl<sup>TM</sup> after 5 days in culture. Maximum attachment  
21 was found with polymer coated with 11% gelatin,  
22 collagen, and collagen in phosphate buffer. Table II  
23 is the attachment of 3T3 cells on polyorthoester,  
24 after 2 days and after 5 days. After 2 days there was  
25 maximum attachment on polymer coated with crosslinked  
26 11% gelatin-11% gum. After 5 days, there was maximum  
27 attachment on polymer coated with crosslinked 11%  
28 gelatin. PH was demonstrated to affect cell  
29 attachment: maximum attachment occurred at pH 7 for 5  
30 days and pH 10 for 2 days. Table III demonstrates 3T3  
31 cell attachment on polyanhydride. Maximum attachment  
32 occurred with uncoated polyanhydride after 2 days.  
33 Materials other than those listed were not studied due  
34 to polymer degradation. Table IV described the

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1 attachment of pancreatic cells (islets and  
2 fibroblasts) on vicryl<sup>TM</sup> after two weeks in culture.  
3 Maximum attachment occurs with polymer coated with  
4 crosslinked or uncrosslinked 11% or 1.5% gelatin and  
5 collagen. Very little attachment of these cells to  
6 polyorthoester and polyanhydride samples was observed.  
7 Table V is the attachment of islet cells after two  
8 weeks in culture, with maximum attachment again  
9 occurring with polymer coated with collagen.

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TABLE I

## 3T3 CELLS ON VICRYL® AFTER 5 DAYS IN CULTURE

<u>Polymer</u>	<u>Attachment</u>
Control (untreated Vicryl® with no cells)	Very little degradation
Untreated	0
Agar (5%)	1
Agarose (6.7%)	1
Gelatin (11%) -crosslinked	2
Gelatin (11%)	4
Gelatin (1.5%) -crosslinked	3
Gum arabic (11%)	1
Gelatin (11%) Gum arabic (11%) -crosslinked	2
Collagen	4
Collagen - phosphate	4
pH 4, 2 days	1
pH 4, 5 days	0
pH 4, 7 days	0
pH 7, 2 days	3
pH 7, 5 days	2
pH 7, 7 days	1
pH 10, 2 days	0-1
pH 10, 5 days	0
pH 10, 7 days	0

Scale

- 0 No viable cells
- 1 Minimal cell attachment
- 2 Moderate cell attachment
- 3 Good cell attachment
- 4 Better cell attachment
- 5 Excellent cell attachment

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TABLE II

## 3T3 CELLS ON POLYORTHOESTER

<u>Polymer</u>	<u>Attachment after 2 days</u>	<u>Attachment after 5 days</u>
Control (untreated, with no cells)	Some degradation	Considerable degradation
Untreated	1	1
Agar (5%)	1	1
Agarose (6.7%)	1	1
Gelatin (11%) crosslinked	2	4+
Gelatin (11%) gum 11%	4	2
crosslinked		
Gum arabic (11%)	1	1
pH 4, 2 days	1	0
pH 4, 5 days	2	1
pH 4, 7 days	1	1
pH 7, 2 days	3	2
pH 7, 5 days	4	3
pH 7, 7 days	2	1
pH 10, 2 days	4+	4+
pH 10, 5 days	0	0
pH 10, 7 days	4	3

Scale

- 0 No viable cells
- 1 Minimal cell attachment
- 2 Moderate cell attachment
- 3 Good cell attachment
- 4 Better cell attachment
- 5 Excellent cell attachment

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TABLE III

## 3T3 ON POLYANHYDRIDE

<u>Polymer Control</u> <u>(untreated, no cells)</u>	<u>Attachment</u> <u>after 2 days</u>	<u>Attachment</u> <u>after 5 days</u>
Untreated	4+	2
Agar (5%)	0	0
Agarose (6.7%)	0	0
Gum arabic (11%)	2	0

Scale

- 0 No viable cells
- 1 Minimal cell attachment
- 2 Moderate cell attachment
- 3 Good cell attachment
- 4 Better cell attachment
- 5 Excellent cell attachment



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TABLE IV  
 PANCREATIC CELLS ON VICRYL<sup>®</sup> AFTER 2 WEEKS  
 IN CULTURE (MIXTURE OF ISLETS AND FIBROBLASTS)

<u>Polymer</u>	<u>Attachment</u>
Control (untreated, no cells)	Little, if any degradation
Untreated	0
Agar (2%)	1
Agarose (2%)	1
Gelatin (11%) crosslinked	4
Gelatin (11%)	4+
Gelatin (1.5%) crosslinked	2
Gelatin (1.5%)	4+
Gum arabic (1.5%)	1
Gelatin (1.5%)/Gum arabic (1.5%) crosslinked	1
Gelatin (1.5%)/Gum arabic (1.5%)	2
Collagen	4++
Collagen - phosphate buffer	3
pH 4, 2 days	0
pH 4, 4 days	0
pH 7, 2 days	1
pH 7, 4 days	2
pH 10, 2 days	1

Scale

- 0 No viable cells
- 1 Minimal cell attachment
- 2 Moderate cell attachment
- 3 Good cell attachment
- 4 Better cell attachment
- 5 Excellent cell attachment

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TABLE V

PANCREATIC ISLETS ON VICRYL® AFTER  
2 WEEKS IN CULTURE

<u>Polymer</u>	<u>Attachment</u>
Control (untreated, no cells)	Very little degradation
Untreated	0
Gelatin (11%) crosslinked	2
Gelatin (11%)	4
Gelatin (1.5%)	3
Collagen	4++
pH 7, 5 days	2
pH 10, 3 days	2

Scale

- |   |                           |
|---|---------------------------|
| 0 | No viable cells           |
| 1 | Minimal cell attachment   |
| 2 | Moderate cell attachment  |
| 3 | Good cell attachment      |
| 4 | Better cell attachment    |
| 5 | Excellent cell attachment |

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1       The method of the present invention is highly  
2 versatile and useful both in vivo and in vitro. For  
3 example, cells on polymer fibers embedded in Matrigel  
4 can be used to create three-dimensional organ  
5 structures in vitro. For in vivo applications, the  
6 polymer structure is tailored to fit the cells so that  
7 the desired function and structure is obtained after  
8 implantation, and so that cell growth, proliferation  
9 and function can be achieved initially in cell  
10 culture. The criteria for successful growth and  
11 implantation is when the transplant demonstrates  
12 functional equivalency to the organ which it is  
13 replacing or supplementing. For example, a functional  
14 kidney would not necessarily have to manufacture renin  
15 as long as it functions as an effective dialysis  
16 apparatus, removing concentrated low molecular weight  
17 materials from the bloodstream. A functional liver  
18 may only need to produce protein such as coagulation  
19 factors and excrete bile. For this purpose the liver  
20 transplant could be implanted in the omentum, the  
21 fatty, highly vasculated membrane adjacent to the  
22 small intestine. A functional intestine should be  
23 able to absorb sufficient nutrients to sustain life.  
24 This could be in the form of caloric solutions rather  
25 than normal "foodstuffs".

26       "Secretory" organs in addition to a liver or a  
27 pancreas can be made by applying the same method of  
28 selecting secretory cells, constructing a matrix,  
29 culturing the cell on the matrix, and implanting the  
30 cell-matrix structure into an area which promotes  
31 vasculature of the cell-matrix structure.

32       As demonstrated in Figure 19, "organs" other  
33 than secretory organs can be made using the method of  
34 the present invention. Nerves may be constructed

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1 using long fibers 52 containing an appropriate nerve  
2 cell 54 to form a nerve structure 56. Following  
3 growth of the nerve along the length of the fiber, the  
4 structure 56 is implanted at the appropriate location  
5 extending from a nerve source to the area in which  
6 nerve function is desired.

7 As shown in Fig. 20a and Fig. 20b, the present  
8 invention can be utilized in patients suffering from  
9 cardiac myopathy. Muscle cells are grown on polymer  
10 spicules (Fig. 20b), which are then embedded on the  
11 surface of the heart itself (Fig. 20a). In accordance  
12 with the previously discussed principles, the damaged  
13 heart itself would not be replaced but new, strong  
14 muscle tissue would grow across the damaged area,  
15 beating in synchrony with the underlying tissue, and  
16 restoring some of the lost function and partially  
17 remodeling the scar tissue.

18 A number of different methods have been used to  
19 create an artificial skin, primarily for use in  
20 treating burn patients. The most successful of these  
21 use a biodegradable matrix of collagen which is seeded  
22 with epithelial cells, attached to the wound site and  
23 overlaid with a moisture impermeable membrane formed  
24 of a non-degradable material such as silicone.  
25 Although these methods are claimed to be useful in the  
26 construction of other organs having a smaller surface  
27 area and larger volume, such as liver and pancreas,  
28 they are not effective when actually attempted. There  
29 is no recognition of the need to provide a high  
30 surface area structure which allows attachment and  
31 proliferation of cells in vitro, prior to  
32 implantation. To be successful, the structure must be  
33 designed to allow adequate diffusion of nutrients,  
34 waste removal, and respiration in the absence of

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1 vascularization. Unless the cells are more or less  
2 equally exposed to the media, with as shallow of a  
3 concentration gradient as possible, this will not  
4 occur. As the cells multiply, the passage of  
5 nutrients, wastes, and gases to and from the cells  
6 becomes limited and the cells farthest from the media  
7 die. Since the artificial skin implants were  
8 immediately placed on the underlying tissue so that  
9 capillary growth into the matrix begins prior to any  
10 significant increase in cell density, this has not  
11 previously been a consideration.

12 The concept of Chimeric Neomorphogenesis hinges  
13 upon the ability of cells to be nourished by diffusion  
14 until vascular ingrowth of the growing cell mass  
15 occurs. It was hypothesized that solid implants of a  
16 cell-matrix configuration using collagen or gelatin  
17 seeded with cells are limited in size by the physical  
18 constraints of diffusion. Others are presently using  
19 complex natural matrices seeded with cells to produce  
20 "organ equivalents". One is a collagen gel that  
21 appears to be a hydrated solution of Type I collagen.  
22 The following experiment tests the ability of this  
23 hydrated collagen to allow diffusion of nutrients to a  
24 cell population.

25 Bovine capillary endothelial cells were plated  
26 in gelatin coated 24 well tissue culture dishes and  
27 allowed to attach overnight. The initial cell number  
28 was  $1 \times 10^5$  cells. The following day the cells were  
29 overlaid with different volumes of collagen Type I at  
30 a final solution of 0.32%. A standard volume of media  
31 was placed over the collagen so that the distance of  
32 nutrient source varied to the cells. The media was  
33 optimized for growth of bovine capillary endothelium.  
34 Dulbecco's minimal essential media, 10% calf serum,

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1 and retinal-derived growth factor at a concentration  
2 of 10  $\mu$ l/ml were used. As depicted in Figure 21a, the  
3 thickness of collagen interspersed between the cells  
4 and the media was 0, 3.0 mm, 5.5 mm, 9.0 mm, and 12.0  
5 mm. At 24 hours, the media and collagen were removed  
6 and the cells were counted.

7 The experimental results, graphed in Figure 21b,  
8 were essentially as predicted. As the thickness of  
9 the hydrated collagen matrix was increased, the cell  
10 viability decreased. Initial cell counts after cell  
11 attachments were  $89,580 \pm 3719$ . Cells adjacent to  
12 media without the interposition of a collagen matrix  
13 doubled in a 24 hour period to  $163,233 \pm 8582$ . A  
14 hydrated collagen gel of 3 mm in thickness between  
15 media and cells resulted in a cell number  $49,587 \pm$   
16  $3708$ . This decreased to  $26,513 \pm 3015$  at 5.5 mm,  $4593$   
17  $\pm 899$  at 9 mm and  $5390 \pm 488$  at 12 mm. All of the  
18 cells at 9 and 12 mm were rounded and nonviable.

19 Figure 22 are photographs demonstrating the  
20 effect of diffusion distance on cell viability and  
21 proliferation: (a) cells from the control well after  
22 twenty-four hours, the cell number having doubled in  
23 twenty-four hours; (b) cells overlayed with 5.5 mm of  
24 0.32% collagen, showing that the cell viability is  
25 markedly diminished and the cell number is far less  
26 than the initial plating number; and (c) cells  
27 overlayed with 12 mm of hydrated collagen placed  
28 between media and cells, showing that all of these  
29 cells are rounded and have died.

30 These data support the concept of diffusion  
31 distance being a critical component of cell viability  
32 and growth for successful implantation. The concept  
33 of uniform cell seeding of a collagen gel is therefore  
34 biologically limited by diffusion distance

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1 constraints. One would expect that an implant of less  
2 than 1 cm<sup>3</sup> would result in cell viability at the  
3 periphery of the implant to a depth of 3-5 mm.  
4 However, the cells in the center of the implant would  
5 not remain viable because of limitation of nutrition,  
6 diffusion, as well as gas exchange. One can envision  
7 large flat gels with very small thicknesses of 5-10 mm  
8 would allow larger implants to occur. However, this  
9 two dimensional solution may have geometric  
10 constraints for implantation. It is also clear that  
11 by increasing cell density, diffusion would be more  
12 limited, and, therefore, the distances would be  
13 commensurately smaller.

14 Although this invention has been described with  
15 reference to specific embodiments, variations and  
16 modifications of the method and means for constructing  
17 artificial organs by culturing cells on matrices  
18 having maximized surface area and exposure to the  
19 surrounding nutrient-containing environment will be  
20 apparent to those skilled in the art. Such  
21 modifications and variations are intended to come  
22 within the scope of the appended claims.  
23

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- 1           1.       A method for controlled cellular  
2   implantation using artificial matrices comprising:
  - 3           a.       providing a matrix formed of a  
4           biocompatible material, said material being  
5           formable into a specific desired shape, and  
6           configured to uniformly support cell growth in a  
7           nutrient solution, having sufficient area to  
8           allow adequate diffusion of nutrients,  
9           elimination of waste, and adequate gas exchange  
10          from the nutrient solution to all of the cells  
11          such that cellular growth and differentiation  
12          can occur both prior to the ingrowth of blood  
13          vessels following implantation and after  
14          implantation as further cell proliferation  
15          occurs.
- 16          2.       The method of claim 1 further comprising  
17   selecting a biodegradable polymer as the matrix  
18   material.
- 19          3.       The method of claim 2 further comprising:
  - 20           b.       providing a population of cells to be  
21           implanted;
  - 22           c.       seeding said matrix with the cells; and
  - 23           d.       growing the cells on said matrix in a  
24           nutrient solution to form a cell matrix  
25           structure.
- 26          4.       The method of claim 3 further comprising:
  - 27           e.       implanting said cell-matrix structure  
28           in a host at a location having adequate  
29           vascularization to allow growth of blood vessels  
30           into said cell-matrix structure.
- 31          5.       The method of claim 4 further comprising  
32   first implanting a matrix formed of a biodegradable  
33   polymer including de-differentiation factors into the



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1 host at the location where said cell-matrix structure  
2 is to be implanted.

3 6. The method of claim 4 further comprising  
4 implanting additional cell-matrix structures having  
5 different cell populations in conjunction with the  
6 first cell-matrix structure.

7 7. The method of claim 3 further comprising  
8 removing lymphocytes from the cell population prior to  
9 seeding.

10 8. The method of claim 3 further comprising  
11 modifying the cells to alter the antigen expression on  
12 the cell surface.

13 9. The method of claim 4 further comprising  
14 selecting cells of a tissue type compatible with the  
15 host's cells.

16 10. The method of claim 1 further comprising  
17 providing compounds selected from the group consisting  
18 of nutrients, cofactors, growth factors, compounds  
19 stimulating angiogenesis, immunomodulators, inhibitors  
20 of inflammation, regression factors, factors  
21 stimulating differentiation and dedifferentiation,  
22 biologically active molecules stimulating lymphatic  
23 network ingrowth, factors enhancing nerve growth and  
24 drugs.

25 11. The method of claim 1 further comprising  
26 selecting the biocompatible material for the group  
27 consisting of polyorthoesters, polyanhydrides,  
28 polyglycolic acid, basement membrane components, agar,  
29 agarose, gelatin, gum arabic, collagen types I, II,  
30 III, IV, and V, fibronectin, laminin,  
31 glycosaminoglycans, and complex mixtures thereof.

32 12. The method of claim 1 wherein the  
33 biocompatible material is selected from the group of

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1 materials which are biodegradable into non-toxic, non-  
2 immunogenic, noninflammatory compounds.

3 13. The method of claim 1 wherein the  
4 biocompatible material is configured as a contoured  
5 disc.

6 14. The method of claim 1 wherein the  
7 biocompatible material is in a fibrous form.

8 15. The method of claim 3 further comprising  
9 selecting the cells from the group consisting of  
10 hepatocytes, bile duct cells, parathyroid cells,  
11 thyroid cells, cells of the adrenal-hypothalamic-  
12 pituitary axis, heart muscle cells, kidney epithelial  
13 cells, kidney tubular cells, kidney basement membrane  
14 cells, nerve cells, blood vessel cells, intestinal  
15 cells, cells forming bone and cartilage, smooth and  
16 skeletal muscle.

17 16. An artificial matrix for controlled cell  
18 growth in a nutrient solution comprising:

19 a biocompatible matrix configured to  
20 provide points of attachment for a cell  
21 suspension, said matrix being configured to  
22 uniformly support cell growth in a nutrient  
23 solution, having sufficient area to allow  
24 adequate diffusion of nutrients, elimination of  
25 waste, and adequate gas exchange from the  
26 nutrient solution to all of the cells such that,  
27 in the absence of a vascular network, sufficient  
28 cellular growth and differentiation can occur to  
29 form a three dimensional cell-matrix structure.

30 17. The matrix of claim 16 wherein the matrix  
31 is constructed from a material selected from the group  
32 consisting of polyanhydrides, polyorthoesters,  
33 polyglycolic acid, collagen, polymethacrylate, silicon  
34 polymers, and combinations thereof.

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1 18. The matrix of claim 16 further comprising  
2 an overlayer enhancing cell attachment.

3 19. The matrix of claim 16 wherein said second  
4 material is selected from the group of materials which  
5 enhance adhesion of cells to the surface of said  
6 matrix consisting of agar, agarose, gelatin, gum  
7 arabic, basement membrane material, collagens types I,  
8 II, III, IV, and V, fibronectin, laminin,  
9 glycosaminoglycans, and complex mixtures thereof.

10 20. The matrix of claim 16 wherein the matrix  
11 is disc shaped and has specifically contoured  
12 depressions for cell attachment.

13 21. The matrix of claim 16 wherein said matrix  
14 is a fibrous structure.

15 22. The matrix of claim 21 wherein said fibrous  
16 structure includes hollow fibers.

17 23. The matrix of claim 21 wherein said fibrous  
18 structure includes solid fibers.

19 24. The matrix of claim 16 wherein said matrix  
20 is biodegradable.

21 25. The matrix of claim 16 wherein said matrix  
22 is configured as spicules.

23 26. The matrix of claim 16 further comprising  
24 compounds selected from the group consisting of  
25 nutrients, growth factors, cofactors, compounds  
26 stimulating angiogenesis, immunomodulators, inhibitors  
27 of inflammation, regression factors, factors  
28 stimulating differentiation and de-differentiation,  
29 biologically active molecules stimulating lymphatic  
30 network ingrowth, factors enhancing nerve growth,  
31 drugs and combinations thereof.

32 27. The matrix of claim 16 wherein said matrix  
33 is configured to provide separate areas of attachment  
34 for cells of different origin.

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1           28. The matrix of claim 27 wherein said matrix  
2 is configured to support growth of tubular structures  
3 within said matrix.

4           29. The matrix of claim 16 comprising separate  
5 areas constructed to maximize attachment and growth of  
6 different cell populations.

1 / 14

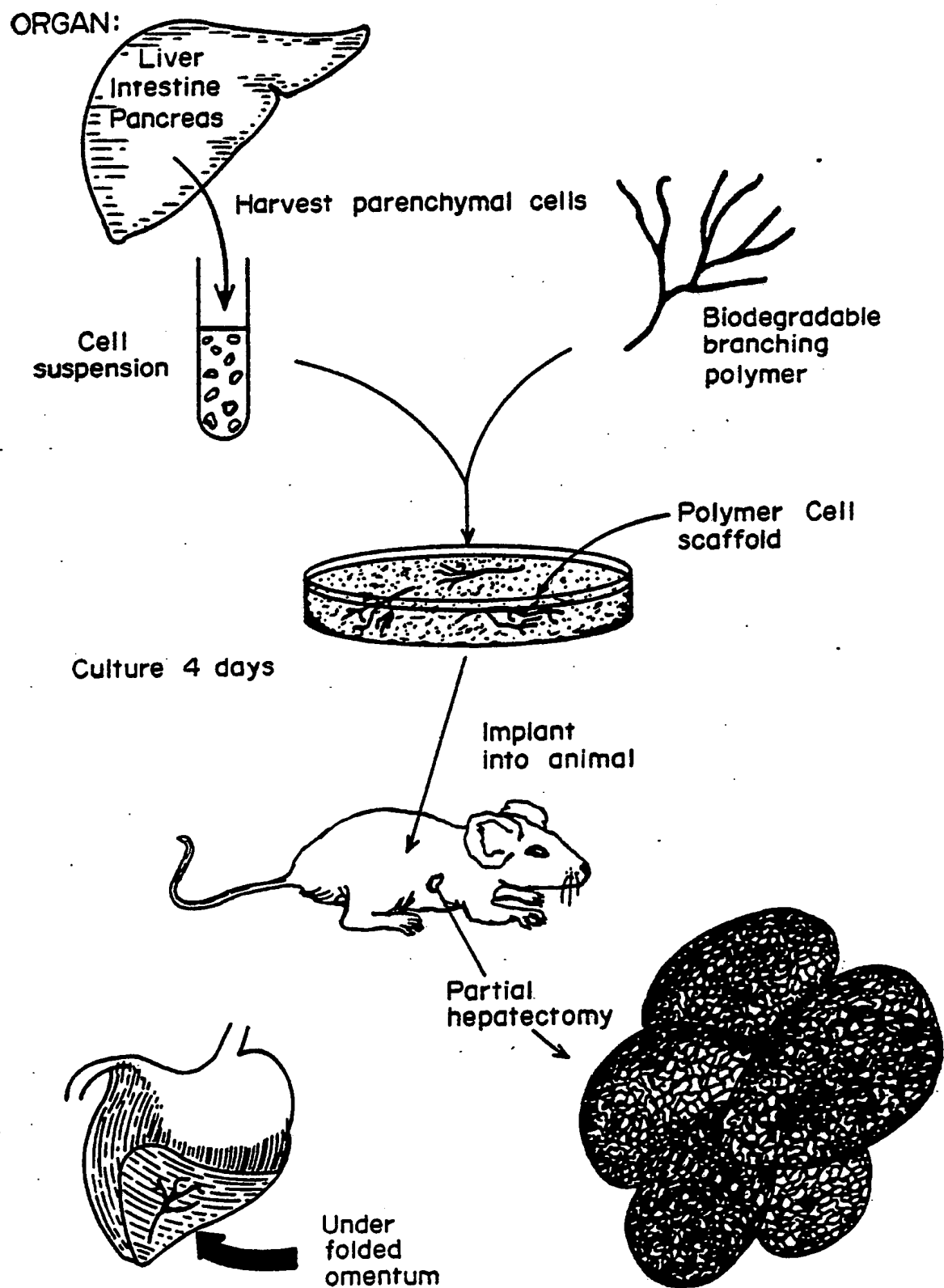
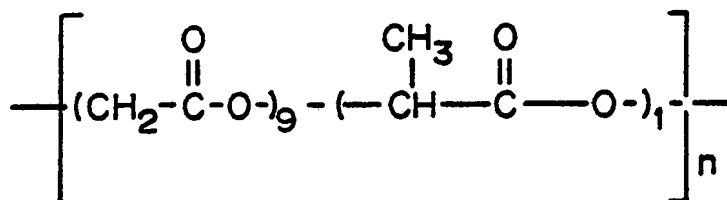


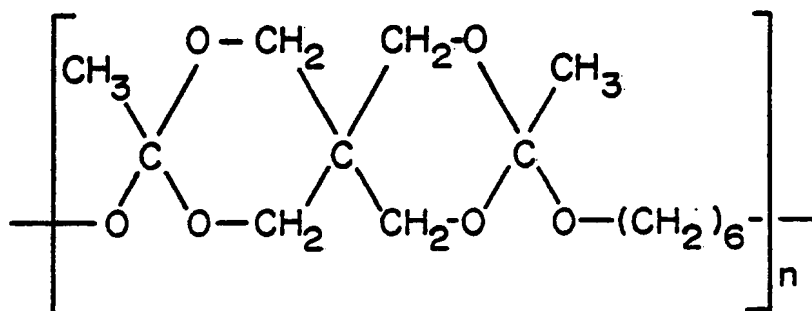
FIG.1

2 / 14

## Polyglactin 910 (Vicryl)



## Polyorthoester



## Polyanhydride

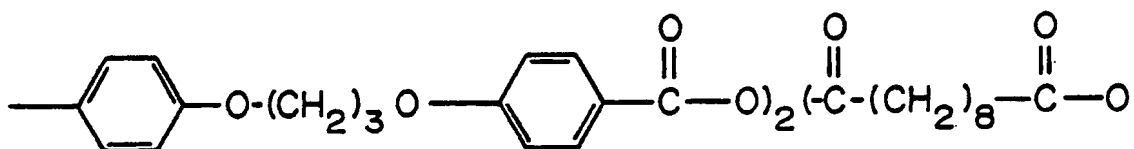


FIG.2

3 / 14

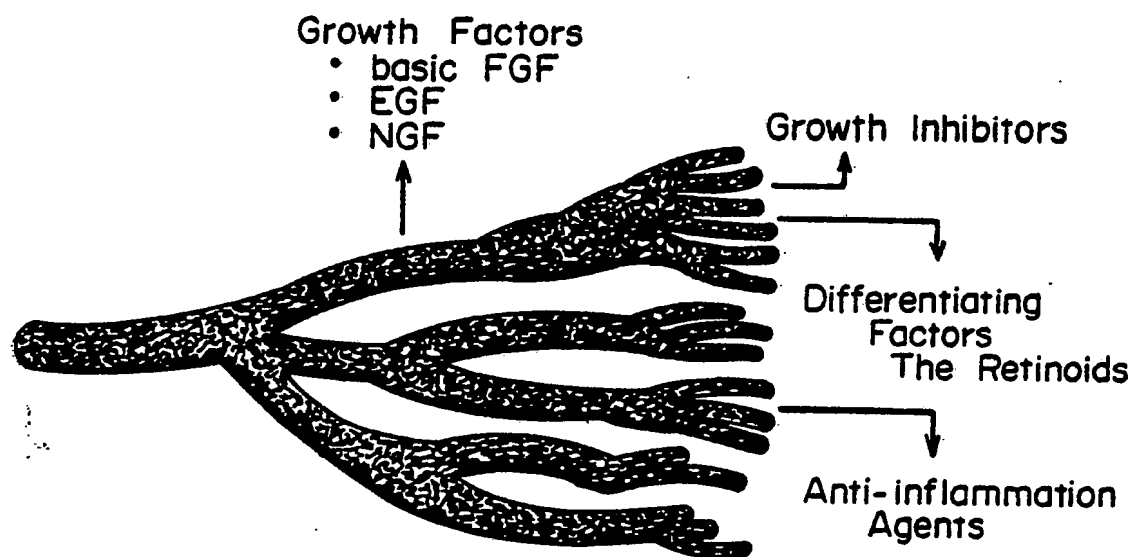


FIG.3

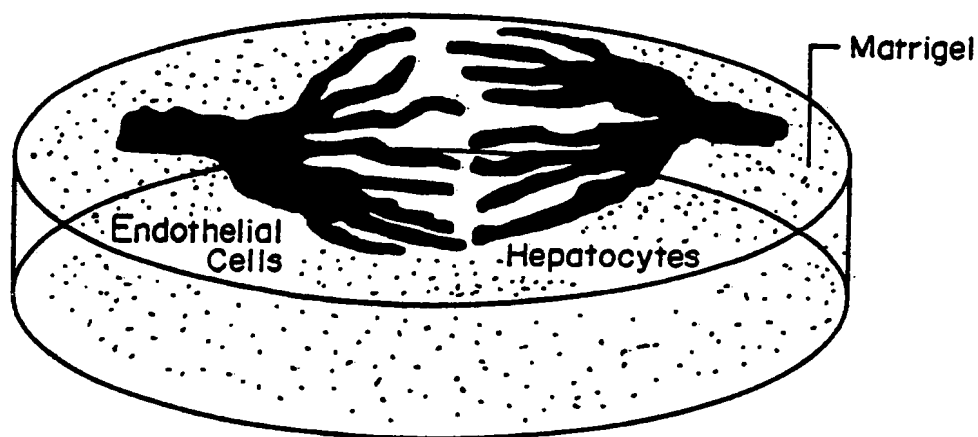


FIG.4

- 4 / 14 -

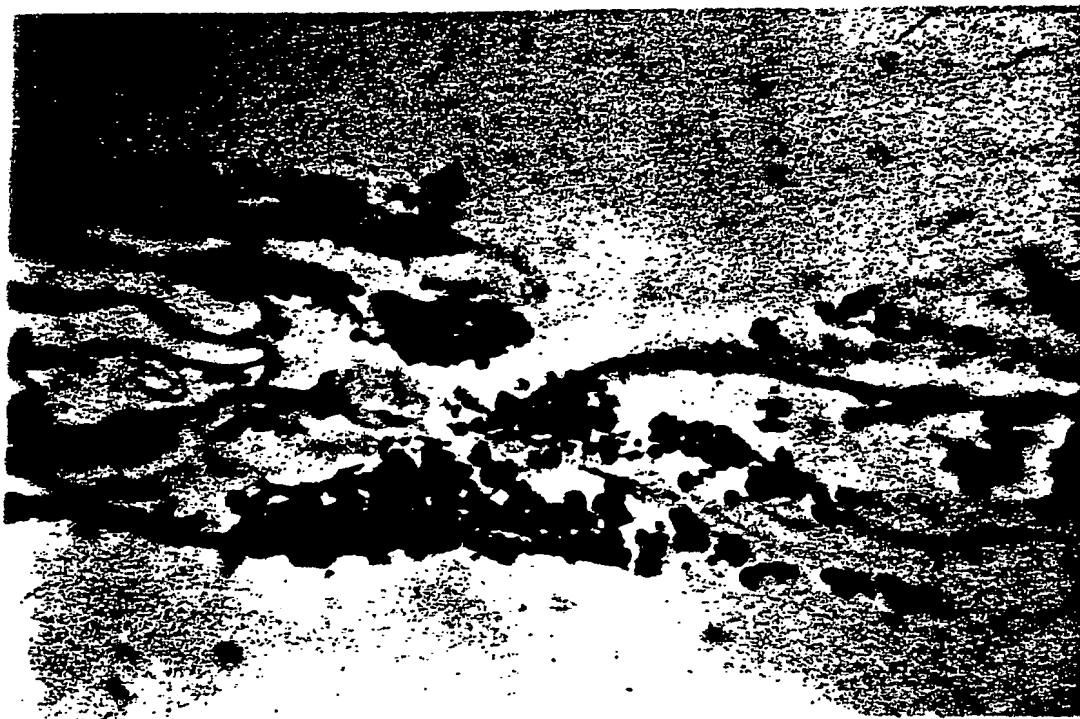


FIG.5

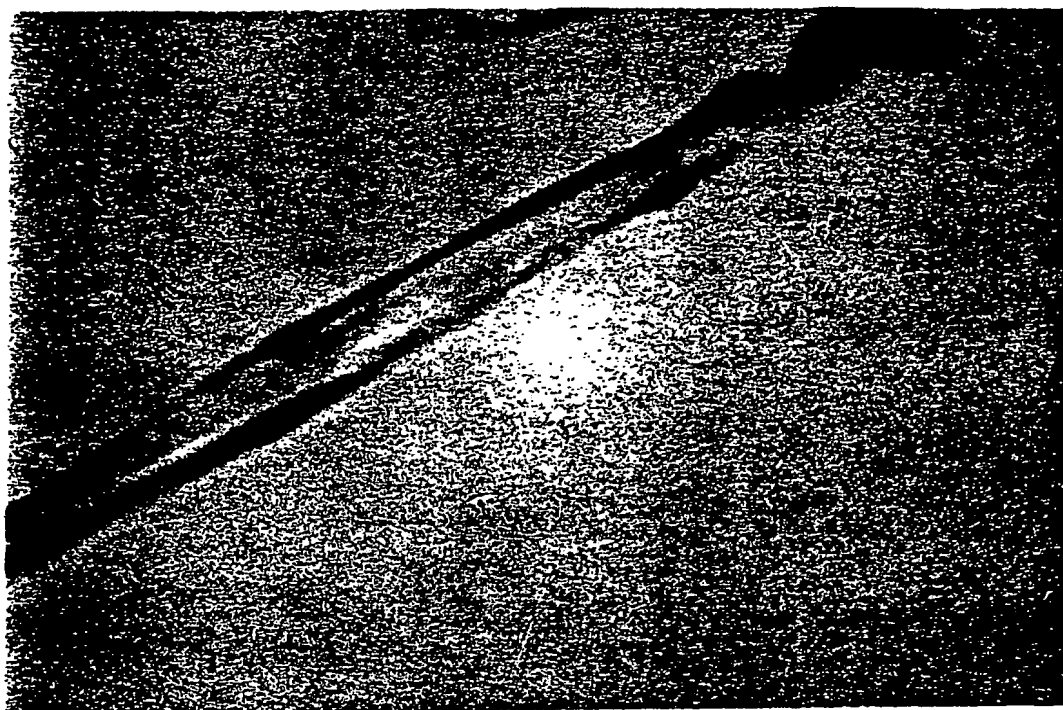


FIG.6



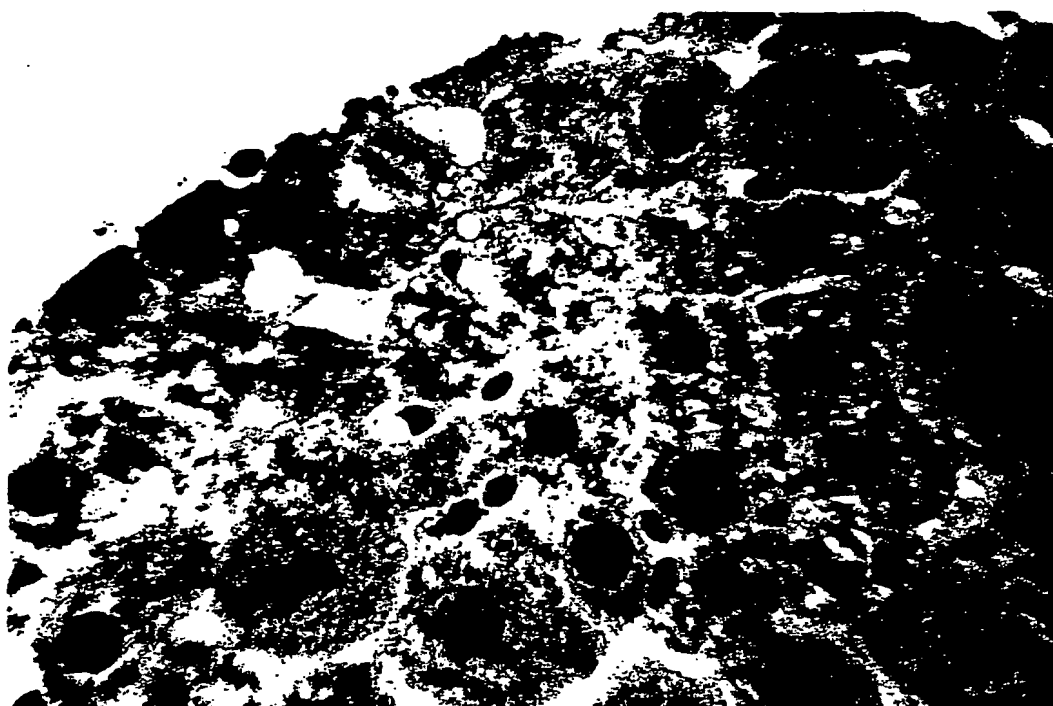


FIG. 7

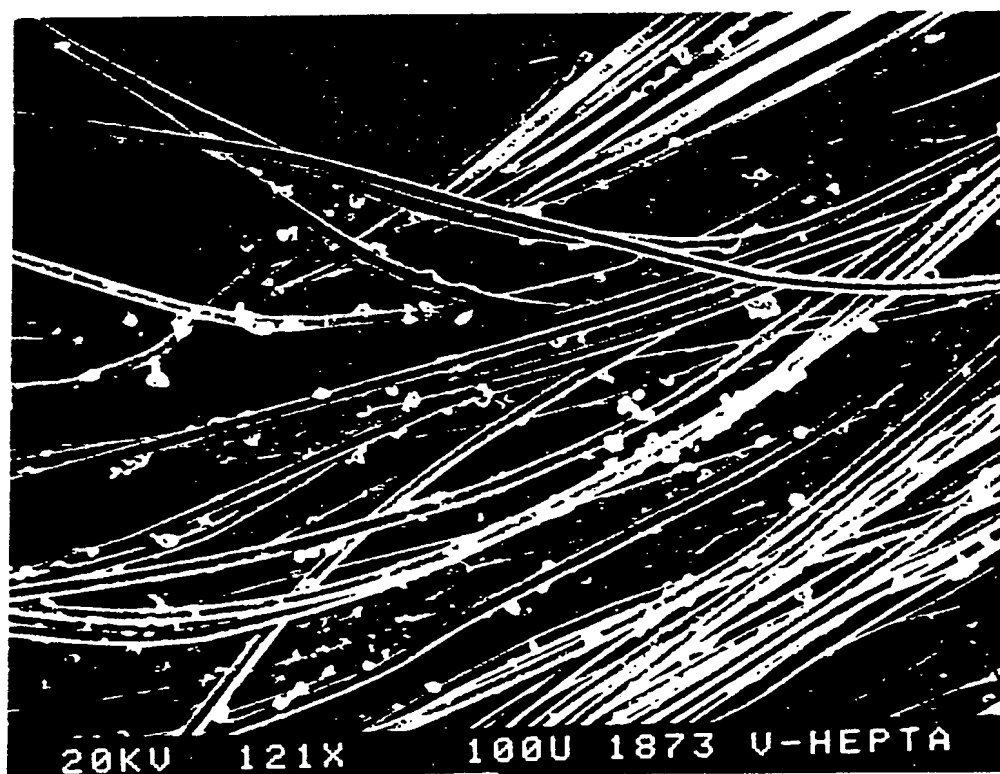


FIG. 8

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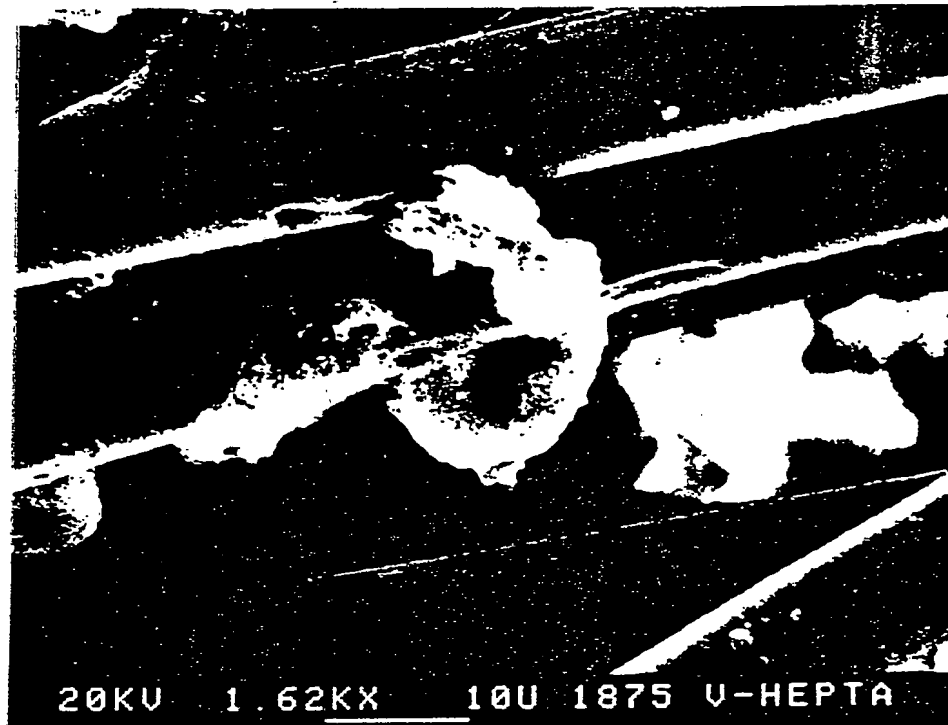


FIG.9



FIG.10

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FIG.11

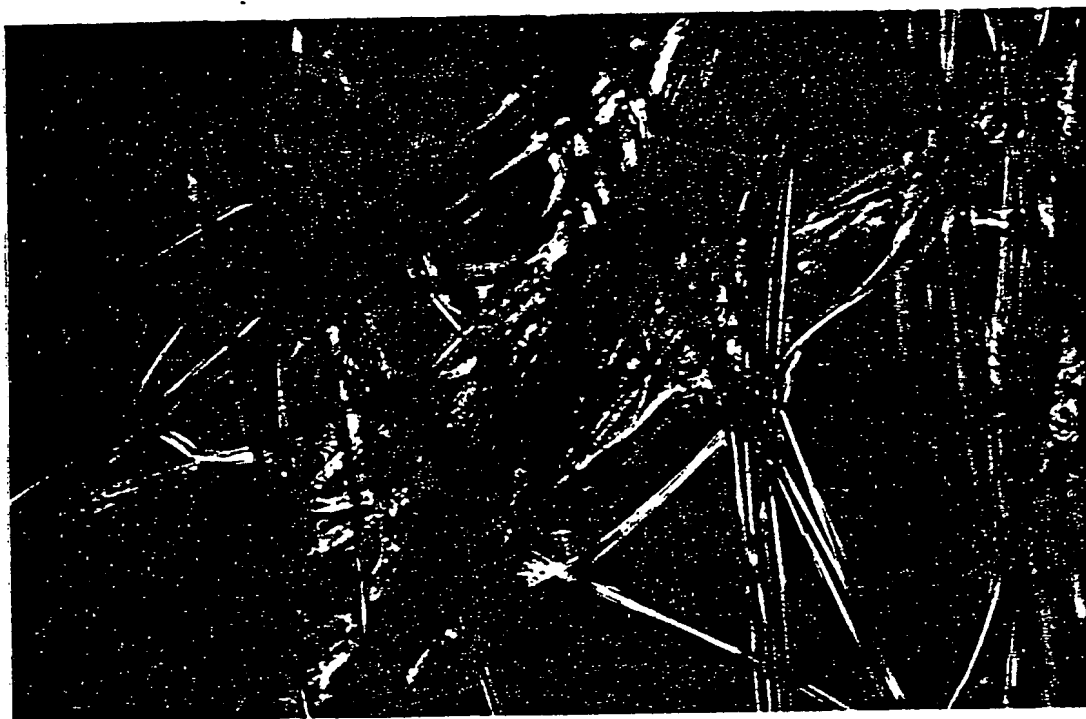


FIG.12

SUBSTITUTE SHEET



FIG.13

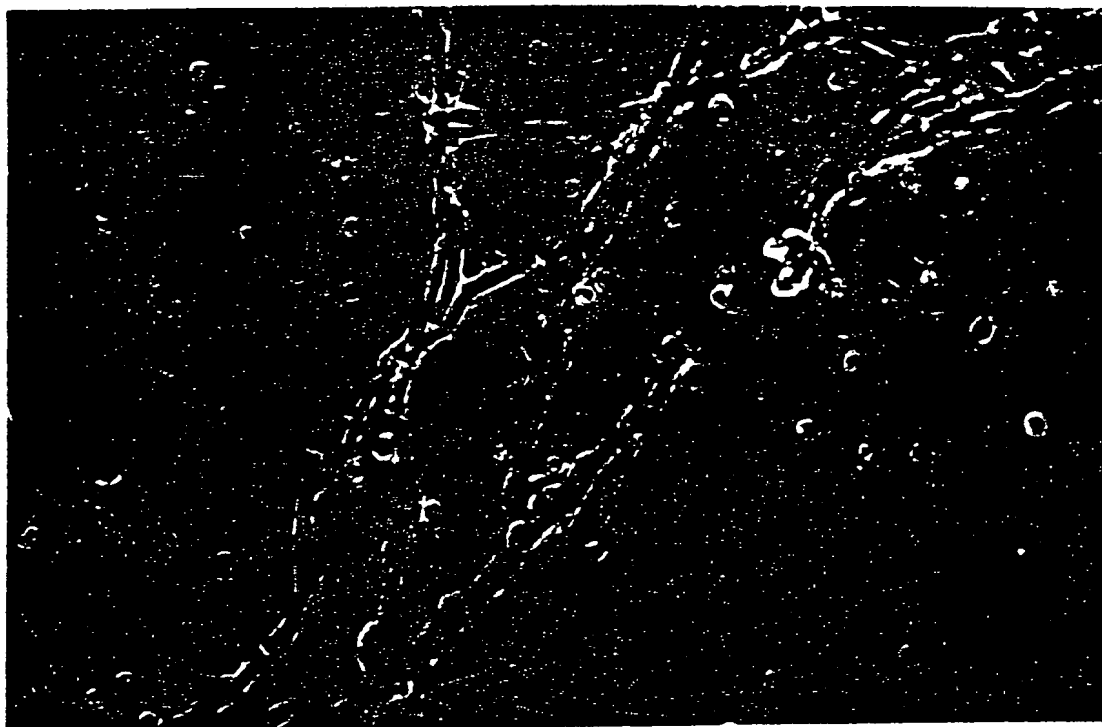


FIG.14



FIG.15

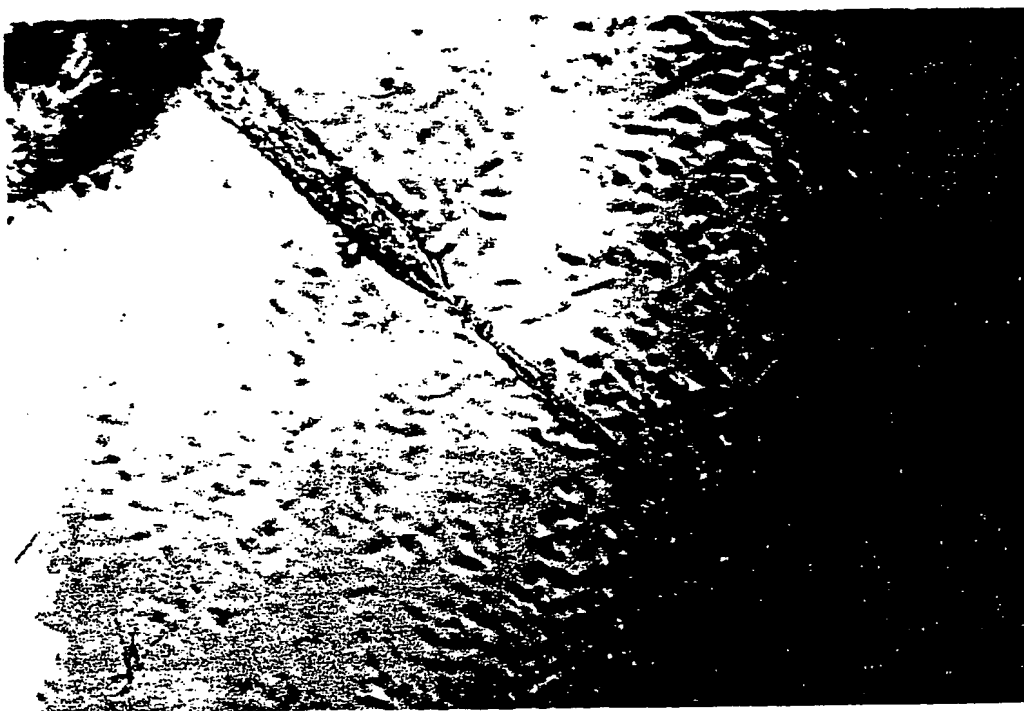


FIG.16

SUBSTITUTE SHEET

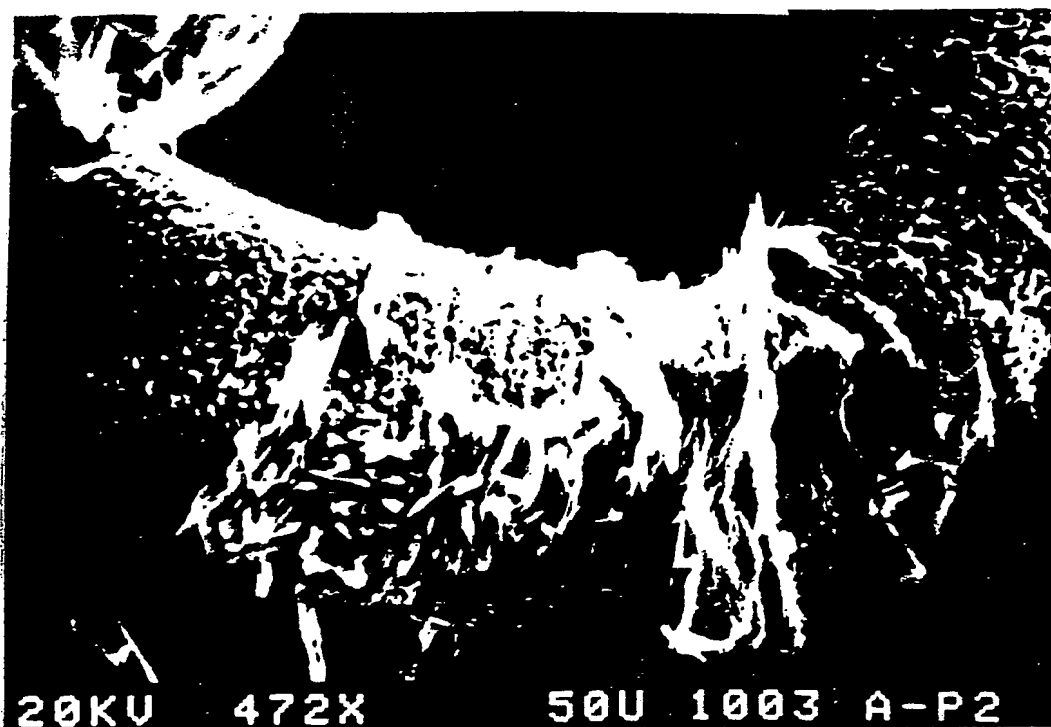


FIG.17



FIG.18

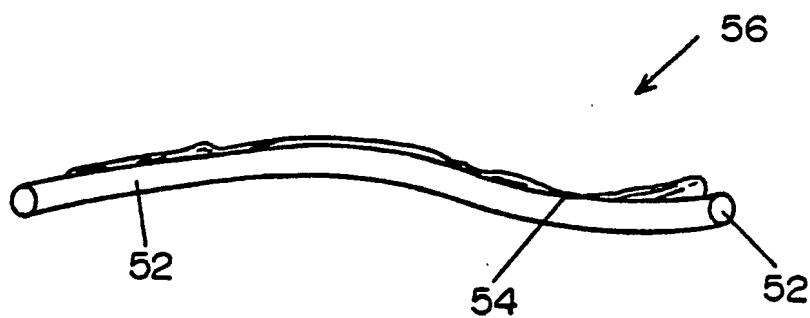


FIG. 19

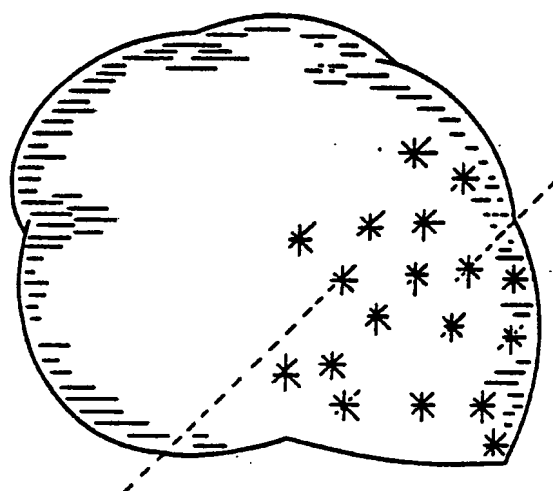


FIG. 20a

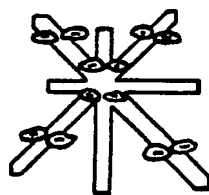


FIG. 20b

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## Nutrition Diffusion Experiment

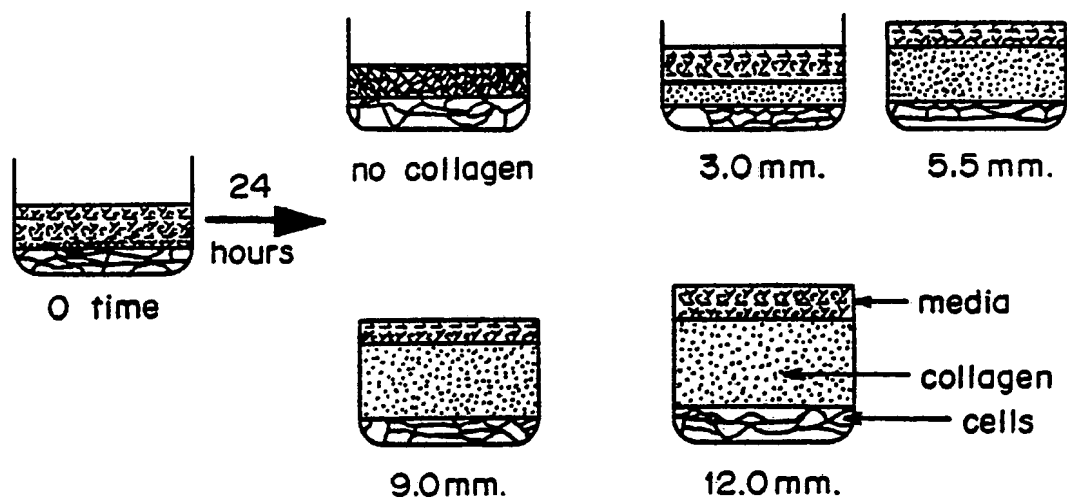


FIG.21a

## Nutrition Diffusion Exp.

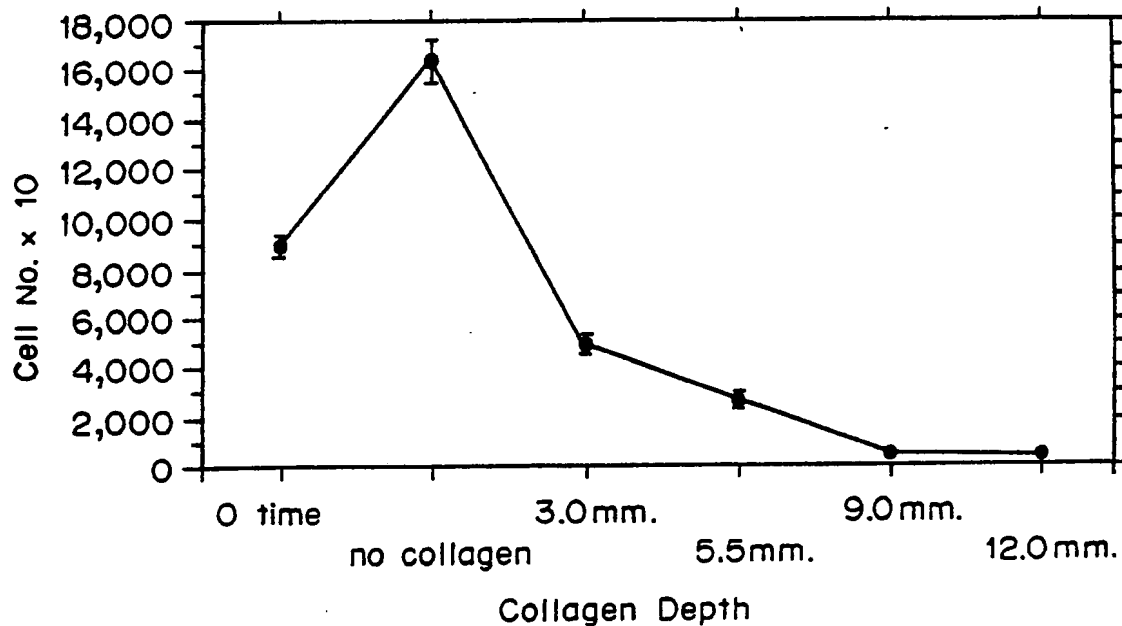


FIG.21b



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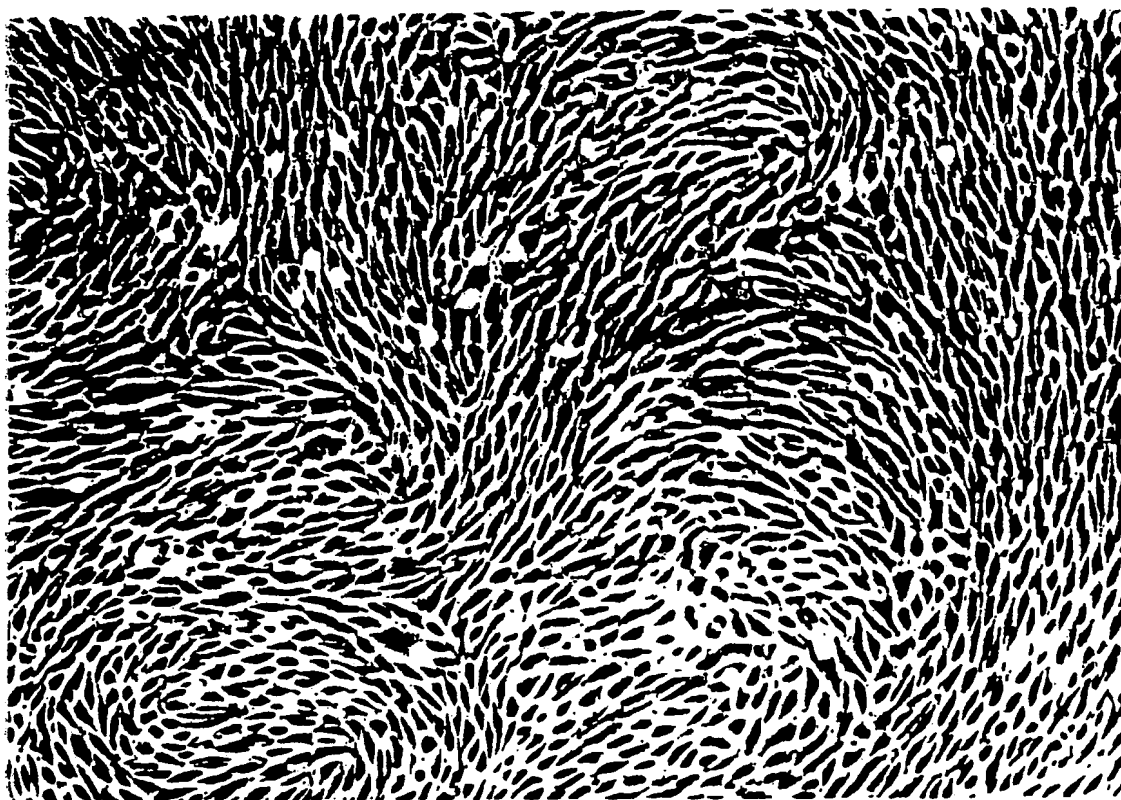


FIG.22a

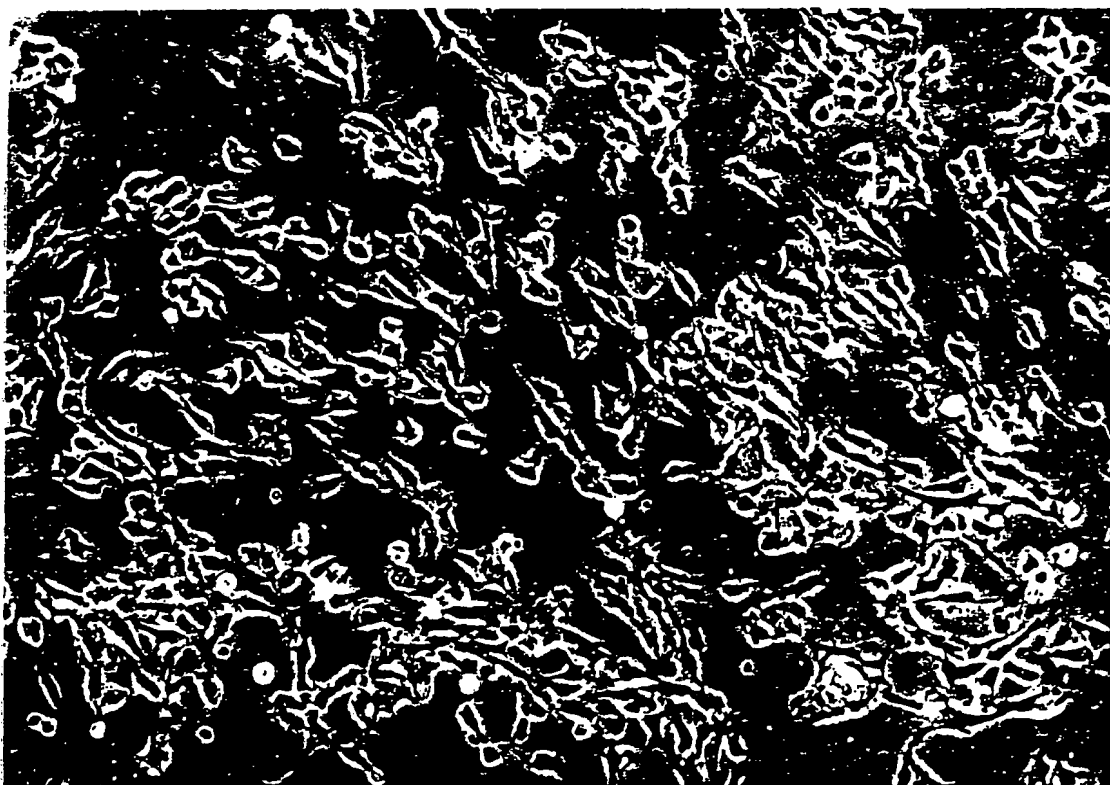


FIG.22b

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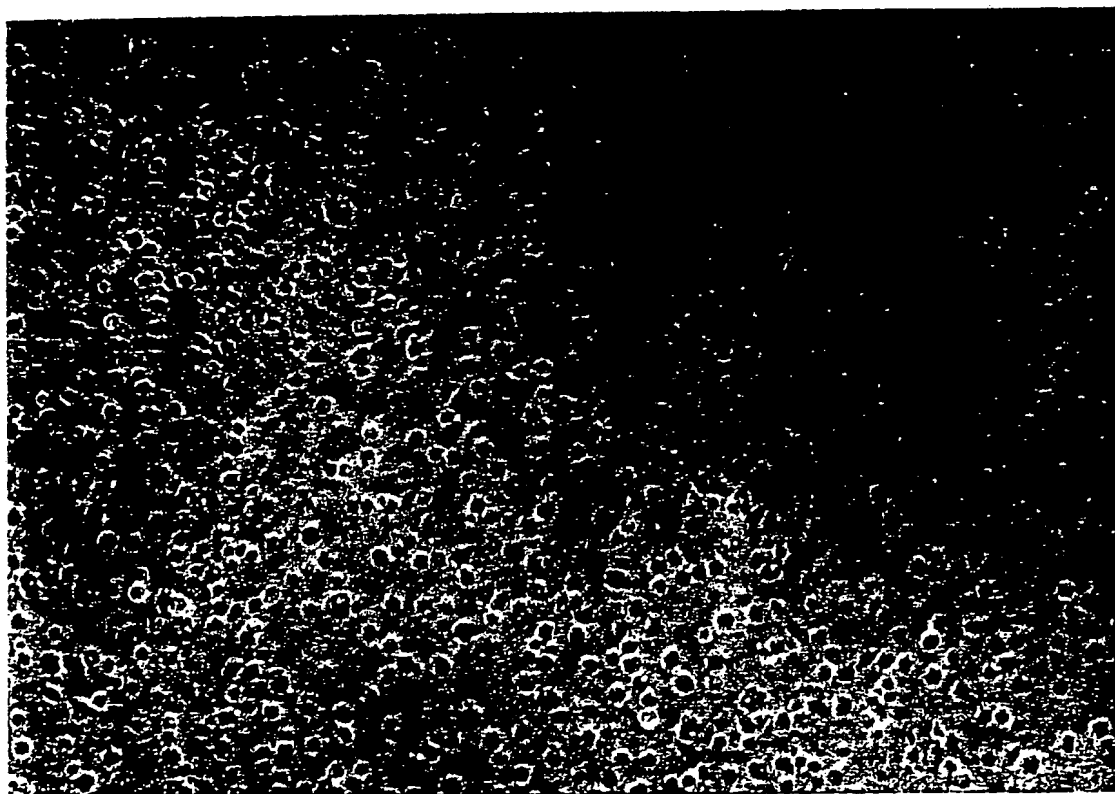


FIG.22c

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/03091

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>1</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>4</sup> A61B 19/00; C12N 5/00 U.S. Cl. 435/240.21		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>6</sup>		
Classification System <sup>1</sup>	Classification Symbols	
U.S. Cl. 128/1R; 435/240.21, 240.23, 240.243; 424/422, 423, 486		
Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in the Fields Searched <sup>5</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup>		
Category <sup>8</sup>	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>14</sup>
$\frac{X}{Y}$	US 4,427,808 STOL, 24 JAN 1984	<u>1-4,9-12,14-17,19,21,24,26</u> 1-29
$\frac{X}{Y}$	US 4,485,097 BELL, 27 NOV 1984	<u>1-4,9-13,15-17,19,21,24,26</u> 1-29
$\frac{X}{Y}$	US 4,458,678 YANNAS, 10 JUL 1984	<u>1-4,8-12,15-19,24,26</u> 1-29
$\frac{X}{Y}$	US 4,060,081 YANNAS, 29 NOV 1977	<u>1,2,11,12,16-19,24</u> 1-29
$\frac{X}{Y}$	US 4,553,272 MEARS, 19 NOV 1985	<u>1,10,16,18,26</u> 1-29
$\frac{X}{Y}$	US 4,559,304 KASAI, 17 DEC 1985	<u>1-3,11,12,16,17,19,24</u> 1-29
Y	US 4,528,265 BECKER, 9 JUL 1985	1-29
Y	US 4,444,887 HOFFMANN, 24 APR 1984	1-29
P, Y	US 4,645,669 REID, 24 FEB 1987	1-29
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p><sup>13</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search <sup>1</sup>		Date of Mailing of this International Search Report <sup>1</sup>
22 MAR 1988		05 APR 1988
International Searching Authority <sup>1</sup>		Signature of Authorized Officer <sup>10</sup>
ISA/US		Catherine S. Kilby Catherine S. Kilby